SOLS CONTAMINÉS

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Résumés

THÈME B

I M P A C T S ET VOIES D'EXPOSITION

SYMPOSIUM B1

A THEORETICAL FRAMEWORK FOR MICROBIAL ECOTOXICOLOGY IN HEAVY METAL POLLUTED SOILS

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POPULATION SIZES AND DIVERSITY OF *RHIZOBIUM* ARE REDUCED BY LONG-TERM HEAVY METAL CONTAMINATION OF SOIL

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Rhizobium is sensitive to heavy metals in soils. During recent years the detrimental effects of soil metal contamination to *Rhizobium* through the long-term application of sewage sludge to land has been well established. The aim of this work was to examine possible changes in the diversity of populations of indigenous *Rhizobium leguminosarum* bv. *trifolii* in response to increasing metal contamination at a long-term sewage sludge experiment at Braunschweig, northeast Germany.

A sewage sludge field experiment was established in 1980 at Braunschweig, Germany on an old arable soil and an old-woodland soil. The following treatments had been applied to both soils with 4 replicates per treatment: 180 kg mineral N ha^{-1} yr^{-1}, 100 or 300 m^3 ha^{-1} yr^{-1} moderately contaminated sludge and 100 or 300 m^3 ha^{-1} yr^{-1} metal - amended sludge for a period of 10 years. Reductions in the numbers of indigenous of *Rhizobium leguminosarum* bv. *trifolii* in plots treated with sewage sludge with total Zn and Cd concentrations below the UK and Ec upper limits have been reported previously.

Rhizobia were isolated from root nodules of white clover (*Trifolium repens*) grown in plant infection tubes and inoculated with dilutions of soil collected from the experiment. Plasmid profile banding patterns of *Rhizobium* isolates were used to differentiate between strains. Plasmid banding patterns were then resolved by agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide. Plasmid banding patterns were identified and the number of isolates with particular patterns established.

Changes in the diversity of rhizobial populations occurred at soil metal concentrations below those causing a reduction in the total population size of rhizobia (Fig 1). The pattern of change in diversity differed between the arable and woodland soil. Such changes or reductions in diversity detected could affect the ability of a population to respond to future stresses that may occur. More research is required to examine the effect of pollutants on the genetic diversity of soil microbial populations. Where detected, changes in genetic diversity may serve as an "early warning signal" before more catastrophic effects on the microbial population occur. The detected changes in rhizobial diversity occurred at soil metal concentrations below the current CEC upper guidelines.

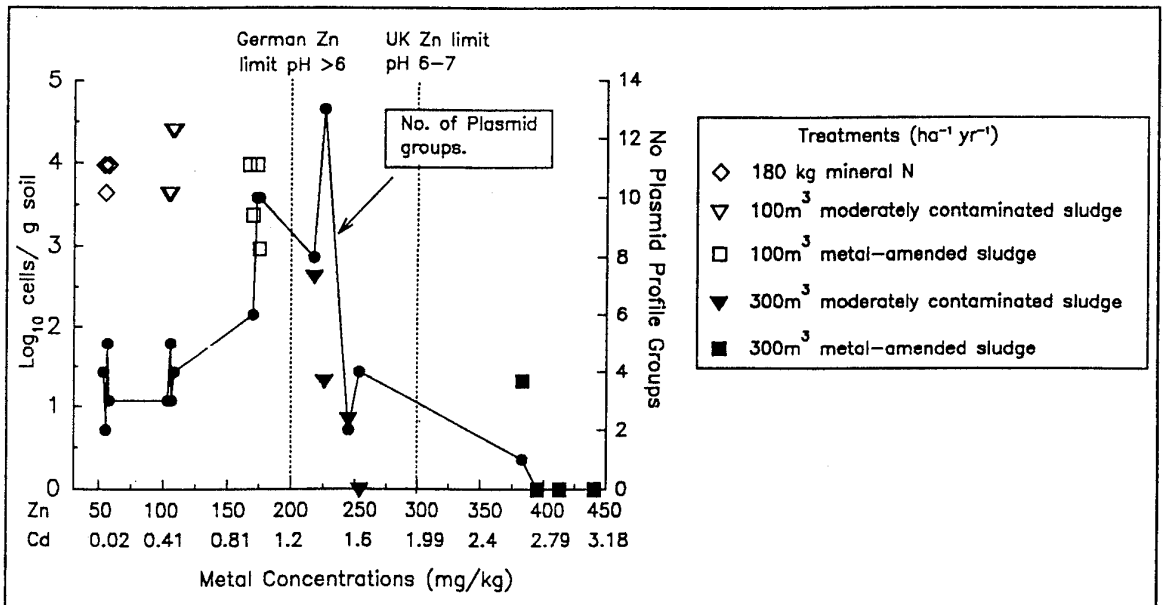

Figure 1: Numbers of plasmid profile groups and indigenous effective *Rhizobium leguminosarum* bv. *trifolii* in soils from the old arable experiment at Braunschweig, Germany. There were relatively few plasmid banding patterns in the least contaminated soils. As the concentration of zinc increased, the number of banding patterns increased even though the numbers of rhizobia were not decreased significantly. Where heavy metal stress increased further, numbers dramatically decreased and a corresponding reduction in diversity occurred. The German and UK limits for zinc concentrations in soils treated with sewage sludge are indicated.

DIVERSITY AND HEAVY METAL TOLERANCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN METAL POLLUTED SOILS

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Although the sensitivity of soil microorganisms to heavy metal pollution has been shown, the long term effect of heavy metals on microbial diversity and microbial function is still poorly understood. Among soil microorganisms, arbuscular-mycorrhizal fungi are ubiquitous in agriculturous soils and some natural ecosystems. They are known to promote plant growth and plant uptake of nutrients. Although at present it is difficult to draw a general conclusion on their ability to mediate or alleviate metal toxicity to plants, they can play an important role since they provide a direct link between soil and plant roots. Further, changes in diversity of mycorrhizal fungi and in their sensitivity to metals can be used to indicate toxic effects of metals in contaminated soils.

Mycorrhizal fungi are isolated directly or via trap cultures with host plants from polluted and unpolluted soils. Long-term field experiments which have been treated with heavy metal contaminated sludges, and soils contaminated through atmospheric deposition from a smelter are used. The number of mycorrhizal spores, and the number of spore types are recorded. Spore germination and early hyphal growth are the initial steps for the establishment of the symbiosis and the propagation of mycorrhizal fungus. Therefore the effect of heavy metals on spore germination and hyphal length is used to compare the tolerance to metals of the different isolates from polluted and unpolluted soils. On the other hand a sensitive *Glomus mosseae* isolate is used as a bioassay to compare the toxicity and bioavailability of metals in a range of contaminated and uncontaminated soils.

Results show that spore density is not always correlated with metal concentrations of the contaminated soils due to different composition (P content, pH, organic matter...) of contaminated and uncontaminated sludges affecting mycorrhizal potential (Weissenhorn *et al.*, 1994). Spore germination decreases with increasing cadmium concentration, but such edaphic parameters as previously mentioned are also affecting the results of the bioassay with the metal sensitive *Glomus mosseae* (Leyval *et al.*, 1994a, 1994b).

Glomus mosseae isolates were found in contaminated and non contaminated soils. The one isolated from polluted soils was more tolerant to Cd than the same species isolated from non polluted soils (Weissenhorn *et al.*, 1993) suggesting an adaptation of the fungi to high metal concentrations.

The actual objectives are to study the diversity of arbuscular mycorrhizal fungi in contaminated and uncontaminated soils and to relate the observed effects to the bioavailable concentration of heavy metals in the soils. Since the taxonomy of AM-fungi based on morphological characters is still uncertain, a variety of different other techniques such as isozyme patterns and new techniques (PCR) are developed to compare and indentify the isolates.

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EARTHWORMS AS BIOINDICATORS OF SOIL COPPER- WHAT MIGHT BE INDICATED?

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Earthworms are an important part of many terrestrial ecosystems. They are a major link within food webs. Their prime role in the maintenance of the physical structure of soil and of favourable conditions for mineralization of organic matter is well appreciated. Interest in their relationship to soil trace elements is documented in over 200 papers on various aspects of the subject. Many of the authors dwell on the potential of earthworms in bioindication of trace elements. The ubiquity of these animals together with a stationary mode of life and a reasonably high biomass make them good candidates for this task. However, generalizations on uptake and efficacy of trace elements and the indicator potential are still contradictory. This is especially true for copper. While some authors consider tissue levels of this element to be efficiently regulated by the animals, others demonstrate a pronounced relationship between soil copper and tissue levels. While populations thrived in soils with up to 1000 mg/kg of copper in some cases, total soil copper concentrations in the range of 50 to 100 mg/kg were suspected to be the prime cause of substantially reduced earthworm numbers in other situations.

Bioindication needs a sound knowledge of these relationships. Experimental data on either copper uptake or toxicity under field conditions are amazingly scarce. Observational data, however, are likely to suffer from interactions not accounted for. Only a sufficiently large data base representative of the various conditions of relevance would be able to minimize these problems. One way of achieving this could be a metaanalysis of hitherto published data. This was done for this presentation with respect to both aspects of indication - accumulation of copper wihtin the worms as well as sensitivity of populations. Information given in tables and figures was compiled in a data base allowing for the main factors that might modify the relationships, as are soil characteristics (pH, CEC, organic matter, ...), species and methodology. For complementing the information from the field, results from laboratory toxicity tests were similarly compiled.

Twenty five papers reporting on copper contents of earthworms from the field and the soil they lived in were found, representing 164 locations. One to five species were analyzed at each of the locations. A pronounced and highly significant dependency of the copper within the worms from total soil copper is revealed by metaanalysis. Up to soil contents of approximately 150 mg/kg the relationship of the logarithmically transformed data is roughly linear with a regression slope of 0.70. Half the variance may be explained thereby. Above this range, data are rather scarce but indicate an upper copper level of approximately 80 mg/kg within the worm tissues (sample means). Higher values have not been measured even in highly contaminated soils (up to some 1000 mg/kg Cu). Scatter around the regression is high. The uncertainty of indication of total soil copper from worm analysis would span a factor of about 1:10. The only soil characteristics reported for a sufficient number of locations ($n = 77$) to be included into the analysis were soil pH, content of organic matter and zinc. Soil pH and organic matter content emerge as significant predictors of worm copper. Considerable scatter remains, however, even after accounting for these factors. Intraspecific differences are inconsistent and not likely to be the main cause of unexplained scatter. The latter also holds for artifacts due to methodological details.

A dozen papers mention densities of earthworm assemblages in relation to copper contamination of soil. The information given is far too heterogeneous to be evaluated in quantitative metaanalysis. Either representativity of sampling is largely questionable or factors likely to interact with soil copper have not been assessed. Nevertheless, cautious generalizations may be extracted. Copper seems to be one of the trace metals most likely to be found in the environment in sufficient amounts to have detrimental effects on earthworm populations. In contrast to lead and cadmium we may find observable effects not only in the nearest surrounding of large metal smelters but also as a result of several agricultural practices. Copper levels below a common threshold of 100 mg/kg may reduce earthworm numbers. This is also substantiated by laboratory toxicity tests. A median NOEC (no observable effect concentration) of only 91 mg/kg results from a compilation of test results on cocoon production of earthworm ($n = 18$). This value is, however, biased towards light sandy soils, where copper availability is presumably high. Under suitable conditions, earthworms may withstand copper concentrations above 1000 mg/kg without observable effects. Unfortunately, toxicity data both from the field and the lab have rarely been linked with either an extensive analysis of chemical speciation of copper in the substrate or tissue levels of copper within the worms. Only this would provide us with sufficient insight to explain some of the wide variation in toxicity levels.

THE INFLUENCE OF CHLORIDE ON CADMIUM UPTAKE BY SWISS CHARD (*BETA VULGARIS* L. CV. FORDHOOK GIANT). I. SOIL EXPERIMENTS

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Recently, high salt (NaCl) concentrations in soil have been identified as the major factor contributing to high Cd concentrations in potatoes in southern Australia (McLaughlin *et al.* 1994). Earlier reports of the effect of Cl on Cd phytoavailability (Bingham *et al.* 1984) were confounded by three factors. Firstly, the Cd concentrations used by Bingham *et al.* (1983, 1984) were high (up to 2.5 mg kg^{-1}), so that much of the Cd was only weakly bound to soil surfaces. Secondly, the counter cations (Na^+ and especially Ca^{2+}) displace Cd^{2+} form surfaces as salt concentrations increased, and increased Cd^{2+} activity in soltion (Chaney 1988). Both these conditions are unlikely to be relevant in agricultural soils where Cd^{2+} activities in soil solution are low and are strongly buffered. Thirdly, osmotic stress under saline conditions may impair root function and allow more Cd to cross the root membrane. The experiment reported here was designed to determine if the effect of NaCl increasing Cd phytoavailability is due to:

(a) displacement of Cd^{2+} into solution due either to ionic strenth effects on sorption or ion exchange of Na^+ for Cd^{2+}, or

(b) increases in total Cd concentrations in solution due to chloro-complexation.

(c) osmotic effects on root function.

Two soils (W, L) were moistened with nutrient solutions containing either NaCl or $NaNO^3$ at concentrations varying from 0 to 120 m*M*. Germinated seeds of Swiss chard (*Beta vulgaris* L., Fordhook Giant) were planted in 1 kg pots and 5 plants were grown until the fourth leaf appeared (15 days in soil W and 19 days in soil L). Plants shoots were harvested, oven dried (70°C) and weighed. Plant material was digested in conc. HNO_3 and Cd concentrations determined by flameless atomic absorption spectrophotometry (FAAS). Soil solution was displaced from the soil by centrifugation at 4,000 RCF for 30 min. Extracted solutions were then centrifuged at 25,000 RCF for 60 min and filtered through a 0.2 µm filter. pH and EC of the solutions were determined immediately. Anions in solution were determined by ion chromatography and cations by inductively coupled plasma optical spectroscopy. Cadmium concentrations in solution were determined by FAAS. To determine if increasing concentrations of Na^+ affected Cd^{2+} activities in solution, the method of Fujii *et al.* (1983) was used to calculated Cd^{2+} activities in soil solutions. Basically, the difference in Cd concentration in soil solution between NaCl and $NaNO_3$ treated soils is calculated. This difference is due to the formation of $CdCl_n^{2-n}$ species. From that difference, the Cd^{2+} activity can be calculated using published formation constants for the complexes and the measured Cl concentration.

Plant weight was decreased by increasing salt concentration, but no significant differences in weights between the $NaNO_3$ and NaCl treatments were observed. Cadmium concentrations in plant shoots and in soil solutions are shown in Table 1. Cadmium concentrations in both plant shoots and in soil solutions were significantly increased by increasing NaCl concentrations, but were unaffected

by increasing $NaNO_3$ concentrations. Cd^{2+} activities in soil solution were unaffected by increasing ionic strength and Na^+ concentrations in soil solution. It is concluded that the effect of NaCl on Cd phytoavailability is due to chloro-complexation of Cd and not due to ionic strength or ion exchange effects on Cd^{2+} activities in solution, or due to osmotic effects on root function.

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Table 1. Mean (±SD) Cd concentrations in plant shoots and in soil solution as affected by NaCl and $NaNO_3$ concentrations in soil solution of two soils (W, upper table, L lower table). Free Cd^{2+} activity data are calculated from the difference in total Cd concentrations in soil solution between Cl and NO_3 treated soils, according to the method of Fujii *et al.* (1983).

SELENIUM UPTAKE AND PARTITIONING IN TOMATO PLANTS IN RELATION TO SULPHATE CONCENTRATION IN SOIL

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The presence of sulphate ion (SO_4^{2-}) in the soil inhibits the accumulation of selenium in the plants either through direct antagonism, since the Se uptake appears to follow the same transport path of SO_4^{2-}, or simply through a dilution effect due to increased plant growth. The interference with sulfur metabolism seems to be the cause of the toxic effects of selenium to plants (Shrift, 1973). The aim of this research was to study the selenium accumulation in tomato plants under low and high levels of sulphate salinity and to investigate on the interactions between selenium and sulphate in various *Lycopersicon esculentum* cultivars.

Five cultivars, E6203, UC82B, PETO81, LA716 and LA2157, grown in pots under protected cultivation, were tested. A selenate concentration of 1.26 μm (i.e. 150 ppb) was used with sulphate concentration of 10 mM (low-sulphate salinity treatment) and 38 mM (high-sulphate saline treatment). The high-salt solution added to the plants was representative of concentrations found in shallow water tables in the west sides of the San Joaquin Valley (California) where the problems of salinity and selenium toxicoty coexist. Plants were harvested 20 and 40 days after transplanting and at fruit ripening. Fresh and dry weights of plant tissues were recorded and the specific root weight (SRW=root weight/total plant weight) was calculated for each plant. Plant tissues were digested with nitric and perchloric acids and following reduction by hydrochloric acid the digests were analyzed by hydride generation atomic absorption spectrophotometry (HGAAS). Selenium uptake was calculated combining randomly the plants of the first harvest with the plants of the second harvest. The total sulphur content in the leaves of the plants of the second harvest was determined using ICP-plasma.

The high sulphate treatment reduced plant fresh weight and specific root weight at the second harvest and it was associated with reduced selenium accumulation in plants, both at the first and the second harvest. The selenium accumulation in the leaves appeared to be more negatively affected by the high sulphate salinity in the soil than in stem and roots. Among the cultivars, E6203 showed the highest growth, but together with PET081, it appeared to be more sensitive to the inhibitive effect of sulphate on selenium accumulation, on fresh and dry weight, and SRW values than UC82B. These results corroborate previous studies which have shown that high concentration of sulphate in the soil reduce selenate uptake by tomato plants and, at the same time, increase sulphur content in the leaves (Shennan *et al.*, 1990).

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EFFECTS OF DECREASED CELLULAR GLUTATHIONE LEVELS ON GROWTH, MEMBRANE INTEGRITY AND LIPID PEROXIDATION IN ROOTS OF COPPER-STRESSED *SILENE VULGARIS*

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When present at toxic concentrations, copper is known to cause plasmamembrane leakiness and lipid peroxidation in croot cells (De Vos *et al.*, 1989, 1993). In view of the oxidative nature of the Cu (II) ion, the cellular antioxidant system, particularly the glutathione (GSH/GSSG) couple (De Vos *et al.*, 1992), might be conceived to play a role in the cellular defence against excessive copper exposure, the more so as toxic copper exposure increases the GSSG reductase activity (De Vos, 1991). Therefore, we studied the effects of pretreatment with buthionine sulfoximine (BSO), which is a potent inhibitor of γ-glutamylcysteine synthase, on the development of early copper toxicity phenomena in roots of copper-tolerant *Silene vulgaris* in hydroponic culture. Control plants were compared to plants pretreated with BSO (500 μM during 5 d prior to the Cu treatments). The parameters measured were the leakage of K^+ from the roots during a 24 h exposure to 100 μM Cu *(cf.* De Vos *et al.*, 1989), as well as the root elongation and the concentrations of GSH, GSSG, phytochelatin-SH (PC-SH), thiobarbituric acid-reactive material (TBA_{rm}) and Cu in the 2 cm apical root segments after a 72 h exposure to a series of copper concentrations, ranging from 1 to 189 μM, which correspond with the highest No Effect Concentration and the lowest EC_{100} for root growth, respectively.

It appeared that BSO-pretreatment reduced the GSH concentration by 60 and 70 per cent without affecting the redox status of the GSH/GSSG couple. As a consequence, the PC-SH concentrations in the root tips after copper treatment were reduced by 35 to 75 per cent, depending on the Cu-exposure level. BSO-pretreatment also slightly lowered the Cu concentration in the root tips (about 20 per cent), but increased the concentration of TBA_{rm}, though only significantly at the highest Cu exposure levels (up to 30 per cent at 189 μM Cu). Remarkably, the BSO-pretreatment was without any effect on the dose-response curves for the effect of Cu on root elongation. BSO-pretreatment did not affect the rate of K^+ leakage from the roots during the first hour after Cu exposure. After 1 h, however, the leakage from the pretreated roots started to increase, whereas that from the control plants steadily decreased.

As argued by De Vos *et al.* (1989, 1991), instantaneous Cu-induced K^+ leakage may be due to direct interaction of Cu (II) ions and membrane sulfhydryls. Lipid peroxidation seems to contribute to the leakage, but probably only after a certain time lag. Therefore, it seems that the BSO-pretreatment effectively increases the rate of Cu-induced lipid peroxidation, which is confirmed by the apparent increase of TBA_{rm}, but fails to interfere with the instantaneous damaging effect of Cu (II) ions on the plasmamembrane. This would mean that neither GSH, nor PCs, can prevent or repair this primary toxic leasion of Cu. In former experiments it was found that the dose-response curves for root growth and for initial K^+ leakage rates were almost identical, suggesting that Cu-induced root growth reduction can be fully explained by the magnitude of this primary instantaneous leasion (De Vos *et al.*, 1989). The absence of any interference of the BSO-pretreatment with the effect of Cu on root growth found in the present experiment suggests that more secundary effects of Cu exposure, e.g. lipid peroxidation, do not substantially contribute to root growth inhibition, at least not in the experimental setting chosen. It is conceivable though, that this could be different in case of a more rigorous lowering of the GSH level.

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METAL AND TREES: IMPACTS, RESPONSES TO EXPOSURE AND EXPLOITATION OF RESISTANCE TRAITS

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Metal resistance traits in trees are reviewed with particular attention to the relative importance of genetic variation versus phenotypically plastic responses to toxic metals. The toxic effect of elevated metals in soils can be readily observed in tree seedlings but, once established, trees appear to be particularly resistant to metal stress. Patterns of metal uptake in trees vary, and elevated concentrations of metals in foliage, bark, roots and woody tissues may not be synonymous with symptoms of toxicity. Knowledge of this variation may have considerable application; inter- and intra-specific variation in resistance and uptake patterns of metals provides a potential opportunity to use trees for biotreatment of contaminated soils. In situations where soil metal levels are elevated by agricultural and industrial activities, the use of short-rotation coppice in bioremediation programmes may allow uptake, harvest and removal of metals. This would be particularly beneficial to future disposal of organic wastes to land, in which metal removal from woodland ecosystems could keep pace with metal inputs. In other situation where soils metal concentrations are highly elevated, and perhaps combined with other edaphic problems, metal removal in this way clearly would be neither realistic nor economically wothwhile. Nonetheless, long-lived trees selected for metal resistance with minimal tissue uptake of metals may provide another solution. A strategy of planting trees for rehabilitation may help to stabilize the site, preveting further deterioration and erosion, and restricting the dispersal of metals to the wider environment. Results of laboratory experiments and from trees naturally established at metal contaminated sites, suggest there is ample justification for field-scale trials to test these hypotheses.

PATTERNS OF SOIL COPPER CONTAMINATION AND TEMPORAL CHANGES IN VEGETATION FOUND IN THE VICINITY OF A COPPER ROD-ROLLING PLANT

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Introduction

This study has examined the patterns of soil copper contamination in the vicinity of a Copper Rod-Rolling plant in Prescot, Merseyside, UK. The plant was established in 1976 on the site of a former domestic refuse tip, with no previous industrial use. The plant manufactures continuously cast copper rod, formed from 99.9% pure copper ingots. Ingots are melted in furnace, and the resultant molten copper is reformed and cast into continous rod. The main sources of emission from the plant is the furnace, measured as emitting copper at a rate of 5.11 mg/m^3, a figure which marginally exceeds the suggested limit of 5.0 mg/m^3 (1).

The plant is surrounded by open grassland (primarily for security purposes), the majority of which was established on imported top soil when the site was initially developed. Some areas have been planted more recently; the latest plantings on the site being made in 1991 (2). Company records indicate the grass mixtures which were sown on each occasion, and the site represents a unique opportunity to evaluate the temporal changes in the responses of a range of grass species to a mono-elemental pollution source. These changes may also be related to copper levels and speciation within the soil profile.

Methods

Soil samples were collected from established grassland at ten points around the factory site. Sites varied in distance from the main source of copper emission (the furnace), and were located in different-aged grassland communities. All soils were sampled to a minimum depth of 45 cm, and at selected sites, samples were collected to a depth of 1 metre. Soil pH was measured, and soil types and textures were assessed. Water- and Nitric acid-extractable copper levels were determined for each profile, and at 3 selected sites, a sequential extraction procedure was followed (3) to determine the fractionation of the extractable soil copper pool.

The current species composition of the grassland was established at 5 of the soil sampling sites, chosen to reflect the full range of sward age and original species sown. At each of the sites, the current sward composition was related to the theoretical composition that would be expected if the original species mixtures had developed. In addition, selected clones from the most recent grassland site, directly adjacent to the furnace, were assessed for copper tolerance, using the root elongation test (4).

Results

The initial soil analyses revealed a pattern of soil copper contamination that appeared to be closely related to the position of the sampling site to the furnace. Nitric acid-extractable copper levels in the upper 20 cm of the soil profiles ranged from 40 - 1115 mg kg^{-1}. Typical

levels found in uncontaminated soils from this area are <20 mg kg^{-1} (5). The most elevated levels were found in soils directly adjacent to the furnace. The distribution of copper is indicated in Table 1.

Table 1. Soil Copper levels (HNO_3-extractable) at grassland sampling sites (values in

Soil depth	n	Maximum	Minimum	Mean
0 - 20 cm	10	1115	40	309.5
20 - 40 cm	10	610	25	221
40 - 60 cm	8	390	35	139
80 - 100 cm	3	120	10	52

Sequential extraction of soils revealed that less than 1% of the extractable soil copper pool was found in the potentially plant-available fraction.

Studies on the species composition of the grassland revealed that great selective pressure had been exerted on the original species sown. In the three oldest communities examined, the species composition had changed between sites over the 19 years since planting. At a site with HNO_3-extractable copper levels of 520 mg kg^{-1}, a healthy sward of *Agrostis tenuis* and *A. stolonifera* had developed, whereas sites of the same age but with lower soil copper content (312 - 434 mg kg^{-1}) possessed more diverse sward structure, with a higher incidence of broadleaved species and moss.

Two younger sites produced strong evidence for the selective pressures exerted by elevated soil copper. At the site sown in 1988 with a meadow mixture (65% grass, 15% *Trifolium repens*, 20% broadleaved sp.), two grass species (*Lolium perenne* and *Poa pratensis*) had disappeared, a further species (*Poa annua*) had colonised extensively, and no broadleaved species except *Trifolium* had survived. The most recently planted site, adjacent to the furnace, was sown with a mixture of four grass species in 1992. Again, two species, *L. perenne* and *P. pratensis*, have virtually disappeared from the sward, with *Agrostis tenuis* and *Festuca rubra* surviving. Soil copper content at this site was >2000 mg kg^{-1}. The sward was composed of large patches of grass, thought to have arisen from tolerant tillers; the seed mixture was a commercial blend, with no inclusion of known tolerant cultivars. Tolerance tests revealed that a) surviving grass at this site possessed copper tolerance and b) larger patches possessed greater copper tolerance.

In conclusion, this site represents a clear example of the rapid evolutionary pressures exerted by a mono-elemental pollution source in less than 20 years. The ability to compare the extant vegetation with that initially planted and to relate this to soil copper content and speciation represents an advance in understanding the potential temporal impact of such emissions.

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SOME EFFECTS OF APPLICATIONS OF HEAVY METAL-CONTAMINATED SEWAGE SLUDGE TO FOREST SOILS ON BIOLOGICAL ACTIVITY IN THE FOREST LITTER LAYER

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Background. The Christchurch (New Zealand) City Council is responsible for a metropolitan sewage system which treats and disposes of sewage from a population of 285,000 plus a wide range of commercial and industrial developments. The current method of sludge disposal is considered non-sustainable, and application of the sewage sludge to local forests has been identified as a possible alternative disposal option. Two trials have been set up to examine the feasibility of this method of sewage sludge disposal and to assess possible environmental impacts including the effects of heavy metal accumulation in the soils.

The sludge disposal trials are located in *Pinus radiata* plantations of various age classes in two forests near Christchurch in the South Island of New Zealand. The soil at Chaneys Forest is a deep sand and that at Eyrewell Forest a shallow silt loam overlying coarse gravels. The soils at both sites are of relatively low fertility. In each forest, experimental plots (30 m x 100 m) were located in 12 and 23 year-old plantings. Within each age class of trees, three different treatments were applied to duplicate plots: (i) control - no sludge applied; (ii) sludge applied to add a total of 800 kg N/ha; (iii) sludge applied to add a total of 1600 kg N/ha. Wet sludge (approximately 8% solids) was sprayed onto the plots using an irrigation spray gun, the sludge being applied in four separate applications over a period of 9 months. The amounts of metals applied in the sludge at the two rates of application were (approximately): Cd, 0.1 and 0.2 kg/ha; Cr, 40 and 80 kg/ha; Cu, 10 and 20 kg/ha; Ni, 1.6 and 3.2 kg/ha; Pb, 7.5 and 15 kg/ha and Zn, 35 and 70 kg/ha.

Objectives and Methods. The objectives of this study were to determine the fate of the heavy metals applied to the forest soils in the sewage sludge, and to examine whether there were any significant effects on biological activity in the soil which could be attributed to the increased metal concentrations.

Forest litter layers (comprising L, F and H horizons plus the sludge residues where present) and underlying mineral soils were sampled from all experimental plots two years after the initial sludge applications (15 months after the final application). After drying and homogenising the samples, heavy metal concentrations were determined using an acid digestion technique followed by flame or graphite furnace atomic absorption spectrophotometry.

Fresh litter samples were also collected from the plots in order to carry out various biological assays. These samples were thoroughly homogenised by hand (any pine needles were cut into 20 mm segments) and the moisture content adjusted before determining (i) biomass-carbon concentrations using a fumigation-extraction method (Sparling *et al.*, 1990), (ii) respiration rates by CO_2 evolution (Anderson, 1982) (iii) urease activity and (iv) acid phosphatase activity using modifications of the methods described by Tabatabai (1982).

Results. Heavy metal analysis of the forest litter layers and the underlying mineral soil two years after the initial sludge applications showed that virtually all of the metals added in

the sludge remained within the litter layer. There had been no significant movement of metals into the underlying mineral soil. As a result, heavy metal concentrations in the litter layer following sludge addition were substantial: Cd, 0.6-2.0 mg/kg; Cr 670-1710 mg/kg; Cu, 140-390 mg/kg; Ni, 26-65 mg/kg; Pb, 150-320 mg/kg; Zn, 630-1290 mg/kg (values expressed on a dry weight basis). The concentrations of some of these metals in the litter layer are far higher than those that have been shown to affect biological activity in mineral soils (McGrath *et al.*, 1994).

The application of sludge with its associated heavy metals was found to have significantly decreased microbial biomass-C concentrations in the litter layer at all but one of the sites examined (Chaneys Forest, 12 year-old trees). Biomass-C in the litter of the sludged plots was less than 50% of that in the controls. However there was no difference between the two rates of sludge application. Litter respiration rates followed the same pattern as biomass-C, with sludge application decreasing respiration rates to less than 50% of that of the control plots. Both litter biomass-C and respiration rates were significantly higher at Eyrewell compared to Chaneys Forest.

Following the initial biomass-C and respiration rate observations, some indicator enzyme activities were determined. Urease and acid phosphatase activity were selected as indicators of nitrogen and phosphorus turnover respectively. In the case of both enzymes, their activity in the forest litter layer was significantly reduced by the application of the metal-contaminated sludge.

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TRACE ELEMENT BIOAVAILABILITY IN PLANTS AND SOILS OF URBAN PARKS (FLORENCE, ITALY)

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Introduction

Urban areas are daily exposed to environmental hazards; emissions produced by industrial plants, power production, vehicular traffic are suspended in the atmosphere, and may accumulate potentially toxic substances in the soils and plants of urban parks.

The aim of this work was to carry out a biomonitoring in the urban area of Florence, Italy, in order to ascertain the presence of some environmentally important trace elements (Cd, Cr, Cu, Ni, Pb, Zn). As study areas, we selected the major public parks (Cascine Park, Boboli Garden, Villa Fabbricotti, Orticoltura Garden), where we suppose that the influence of vehicular traffic and anthropogenic contamination are reduced.

The interest was focused on three items:

a) to know the background level of trace elements in soils of urban parks of Florence, and their bioavailability;

b) to determine the amounts of trace elements in plants of urban parks;

c) to assess if the differences recorded are a consequence of the location of the areas investigated or are caused by environmental pollution.

Materials and Methods

The topsoil (A-horizon) and the leaves of the most representative trees in the major urban parks in Florence (*Laurus nobilis* L. in Boboli Garden, Orticoltura Garden and Villa Fabbricotti; *Celtis australis* L., *Platanus orientalis* L., *Quercus robur* L., *Aesculus hippocastanus* L: and *Tilia platiphillos* Scop. in Cascine Park), were collected over a complete vegetative cycle.

Total element concentrations in the topsoil were determined by AA spectrometry, after digestion of the < 2 mm fraction with $HClO_4$ + HF + HCl;

EDTA-extractable element concentrations in the topsoils were determined by AA spectrometry;

Plant leaves, previously washed with distilled water, were powdered and solubilized with HNO_3 conc.; trace elements in leaves were determined by AA spectrometry.

Results and Discussion

The levels of trace elements in the soil are generally under the attention threshold proposed by Tiller (1992), with the exception of Pb (225 mg/kg), Ni (110 mg/kg), Cu (105 mg/kg) in Villa Fabbricotti and Cascine Park.

As regards the plant-available elements (EDTA-extracted) in the topsoil, levels up to the attention threshold are recorded for Cu (27 mg/kg), Zn (55 mg/kg), Pb (197 mg/kg) at sites close to areas with heavy vehicular traffic, especially in the Boboli Garden.

The elemental concentrations in plants are generally within the range of normal values proposed by Kabata-Pendia & Pendias (1984), with the exception of Cr (3.2 mg/kg) and Pb (10.9 mg/kg) at Villa Fabbricotti and Boboli respectively.

In the leaves of *Laurus nobilis* L., the most diffused species in the areas studied, the levels of Cd, Cu and Cr are systematically higher in Villa Fabbricotti and Orticoltura Garden than in Boboli Garden, whereas Pb levels are remarkably higher in Boboli Garden, probably because of the location of the parks, their history and menagement.

The trace element distribution in plants over the vegetative annual cycle shows different trends. Some elements, like Cd and Pb, show accumulation phenomena at the end of the vegetative cycle (November), while Cr, Cu, Ni and Zn are accumulated at the starting cycle (April), as a consequence of their geochemical mobility and the plant metabolism, irrespective of the species considered.

The Biological Absorption Coefficent (BAC) was calculated for samples from the Cascine Park, Villa Fabbricotti and Orticoltura Garden in order to relate trace element levels in soils with plant uptake. The BAC values for the elements considered are higher than those quoted in the literature (Brooks, 1983). Moreover, a different behaviour between essential and non essential elements is recorded, since the former (Cu and Zn) show BAC values higher than the latter (Ni, Cr, Cd, Pb), and their uptake seems to be controlled by plants.

A spatial variability was also observed in trace element levels of both soils and plants of major parks (Cascine and Boboli). This differential distribution may be related to anthropogenic contribution both direct (vehicular traffic inside the parks) and indirect (aerial currents from areas close to the parks).

The results obtained suggest that trace element monitoring is an important tool in assessing the environmental degradation of urban areas.

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PLANTS AS BIOMONITORS OF POLLUTION

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Indicator plants which are diagnostic of particular environmental conditions have been used since the sixteenth century by mineral prospectors. Vegetation may reveal the presence of man-made contaminated land in similar ways: visible symptoms of physiological disorders (e.g., chlorosis); distinctive indicator vegetation; high concentration of metals in tissues; high tolerance or low toxicity indexes (effect parameters); changes in physiological parameters (biomarkers).

The most widely used indications of contaminated soil have been visible signs: bare patches, chlorotic symptoms and distinctive vegetation. The most pronounced effects on vegetation are produced as a result of mining and industrial activities, where in addition to high metal concentrations, there are deficiencies of macronutrients.

A number of higher plants species have been suggested for use as passive bioindicators. These include: *Populus nigra* "italica", the Lombardy poplar *Pinus sylvestris*, Scots Pine and *Taraxacum officinale*, dandelion. Unlike the traditional indicator plants, these have widespread availability and occur frequently in both agricultural and urbanised areas.

In the UK, areas of metal-rich soils often reflect release from mining activities superimposed on natural inputs from bedrock sources and bedrock materials. The most important mining areas were Devon, Cornwall, central and north Wales, the Pennines, and the southern uplands of Scotland. The once numerous mines are almost all abandoned for economic reasons, and in many of these old sites indicator plants, including *Viola lutea*, *Armeria maritima*, *Minuartia verna* and *Thlaspi alpestre*, reveal the presence of high concentrations of metals. In addition numerous other plant species including heather, *Calluna vulgaris*, bramble, *Rubus ulmifolus* and gorse, *Ulex europaeus* colonise areas in many of these sites.

The factors influencing the uptake and tolerance of plants on old contaminated sites is at present under investigation. Some results are presented here for the indicator plant, *Armeria maritima* growing on a number of such sites, these include a copper impregnated bog, a salt marsh, a contaminated estuary, old copper mines (Botallack and Levant), old arsenic mine and works (Poldice) and an uncontaminated maritime area. Concentrations of elements have been determined in soils, roots and leaves plant/soil concentration factors (CF) have been determined, some results are presented in Tables 1 and 2.

Table 1. Mean Concentration Factors CF1 (leaf/soil concentration ratio) and CF2 (root/soil concentration ratio) for Armeria maritima *from contaminated sites.*

	Bog		Botallack		Levant		Poldice	
	CF1	CF2	CF1	CF2	CF1	CF2	CF1	CF2
As	0.007	0.052	0.01	0.003	0.003	0.01	0.012	0.009
Cu	0.015	0.99	0.025	0.092	0.029	0.156	0.028	0.116
Zn	0.53	1.075	0.22	0.20	0.12	0.24	0.55	0.64
Cd	0.37	1.19	0.30	0.92	0.2	0.094	0.50	0.52
Pb	0.018	0.16	0.010	0.068	0.006	0.05	0.005	0.023

It can be seen that at the top site copper, zinc and cadmium are concentrated in the roots, and the concentration factors are consistently higher than those for the other sites, this may reflect the soil conditions: acidic, waterlogged and high carbon. Arsenic is largely excluded from the roots in all sites, that which is taken up remains in the roots and little is translocated to the leaves. Similarly copper and lead remain largely in the roots. Zinc and cadmium show similar concentrations in both roots and leaves showing translocation throughout the plant.

Table 2. Ranges and Geometric Means (GM) (mg/kg) of elemental concentrations in soils from contaminated sites.

	Bog Range (GM)	Botallack Range (GM)	Levant Range (GM)	Poldice Range (GM)
As	13-39 (18.4)	66-494 (219)	140-2875 (672)	432-37600 (3450)
Cu	1035-7620 (3635)	290-6505 (1891)	125-2535 (665)	160-4780 (961)
Zn	95-255 (120)	110-350 (228)	25-790 (234)	47.5-1860 (297)
Cd	0.95-3.5 (1.3)	0.5-2.5 (1.4)	0.2-2.5 (1.2)	0.4-60 (1.7)
Pb	4-85 (28.5)	55-235 (139)	15-195 (103)	59-1500 (273)

The results indicate that although 'total' concentrations some elements in soils were very high (e.g. Cu in the bog, 1035-7620 mg/kg; As at Poldice, 432-37,600) these concentrations may not always be revealed in the aerial parts of the plant. Many environmental factors influence the uptake of metals by this and other species.

INSTRUMENTAL MULTIELEMENT ANALYSIS OF ENVIRONMENTAL SAMPLES

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Instrumental techniques available for environmental analysis have reached a high degree of sensitivity for most of the chemical elements of the periodic table. The ppm and upper ppb ranges are a domain for highly specialized laboratories especially equipped with clean room conditions, quartz tools etc.

Within modern ecosystem research it may be assumed that interrelations between organismic activity and flow rates as well as flow patterns of the elements, arise from the various components and compartments of an ecosystem which can only be correctly interpreted with analytical coverage of, if possible, all elements.
Undoubtedly, the nature and extent of this facotrs such as weather factors. Consequently the flow rates and flow patterns vary as a function of these factors. This must always be remembered when interpreting ecochemical data. The results are only characteristic of the factor constellation prevailing during sample collection. The preliminary history of the sample material should also be included in a description of the factor constellation.

Depending on the components of an ecosystem to be investigated, how often sampling took place and the possibility of making comparisons with other ecosystems of the same type, the following interpretation possibilities arise for the data material (Fig. 1).

a) If the components and compartments studied in the system are immediately consecutive stages of the material flow then statements can be made on the basis of a multielement analysis concerning

- the concentrations in which individual elements occur,
- whether and to what extent individual elements occur in a correlated manner in the samples studied above,
- whether the samples studied display an accumulative, indicative or rejective behaviour for certain elements.

b) If a multielement analysis is carried out for a certain factor constellation with several ecosystems of the same type (similar soil conditions and plant composition) subjected to a different input of elements then a comparison of the data will provide initial insights into how differently comparable ecosystems may react to various material inputs. Not only simple changes in concentration ratios have to be considered here but also changes in the accumulative behaviour of individual plant species, shifts in element correlations etc.
Traditionally particular interest is attached to a consideration of those elements whose ecotoxicological significance is known.

c) The results of multielement analyses for a factor constellation recognized as typical of an ecosystem are able, if compared with systems displaying different plant composition and soil conditions, to form a reliable basis (possibly together with other data) for a consideration and causal analysis. This provides indications of

- whether, to what extent and under what conditions the element correlations in the individual matrices change,
-whether, to what extent and under what conditions changes in accumulative behaviour are to be directly attributed to different soil conditions or plant-specific element patterns,
- the factor constellations under which and to what extent concentration changes may be observed.

d) If the results of multielement analyses are compared over a lengthy period of time for similar ecosystems only differing with respect to the material input into that system then conclusions about the stability behaviour of the system may possibly result from the aspect of the material flow.

The experimental plant sketched above and reproduced in Fig.1 shows that ecosystem multielement analyses should not be equaled with the simple measurement of element concentratons. The validity or interpretability of concentration data within a series of measurements should always be regarded in view of the question in hand and the special parameter constellation. From the biological point of view attention was focused on the concepts of concentration, correlation, accumulation, rejection and indication of individual elements or groups of elements, and from the analytical aspect specific problems concerning appropriate sampling, sample preparation and the detection sensitivity of individual instrumental methods.

As one of the results a Biological System of the Elements (BSE) based on data for terrestrial plants (glycophytes) was established, which preliminary considers aspects of basic biochemical and physiological research (Fig. 2). This includes: (a) the interelement relations of single elements within an individual expressed as a linear correlation coefficient, (b) the physiological functionality of single elements paying attention to evolutionary development during the emergence of organic life from the inorganic environment, and c) the uptake form of individual elements and their compounds by the living organism.

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3ème page

GEOCHEMICAL FACTORS INFLUENCING Cu, Pb AND Cr CONCENTRATIONS IN URBAN SOILS AND PLANTS

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The objective of this study was to evaluate the influence of human activity on trace metal concentration in urban soils and the subsequent plant uptake.

Lublin is a city with about 400 thousand inhabitants. Bystrzyca River divides the city area into two morphologically and petrographically different regions. Soils on the west side of the river were developed from a loess. On the east side of the river, there is a mosaic of shallow layers of silty sands and silty loams overlying a limestone bedrock. Air pollution, from a variety of sources, is the main contributor to the soils contamination with trace metals. Emissions associated with coal combustion both in electric power plants and in private houses are one of the main sources of air pollution. Lead contamination is thought to be derived mainly from the combustion of leaded gasoline by motor vehicles. Lublin's metallurgical plant is another significant source of metal contamination. Particle fallout in the densely populated and industrialized parts of the city was 250 Mg km^{-2} $year^{-1}$ while in suburban areas it was about half this value.

Samples were collected in July 1982 from the 0-20 cm soil layer. At the same points, the above-ground sections of 7 individual *Capsella Bursa-pastoris* plants (a widespread ruderal plant) were collected. The sampling area was divided into two zones. The suburban zone is partly residential and partly agricultural in nature. It is characterized by relatively low population density. The downtown zone is characterized by business, residential or industrial activities and contains the main roads. A total of 29 samples were collected; 16 from the suburban zone and 13 from the downtown zone. Plant samples were rinsed in deionized water for about 30 s, dried at 100°C, ground and then a representative 1 g sample was ashed at 500°C in a muffle furnace. Soil samples were air dried, sieved through a 1 mm screen and ground. Organic carbon and pH in water were determined. Fallout samples were collected at several sites in the downtown zone during a one year period as a part of a routine sampling program performed by a specialized government agency. Copper, lead and chromium were determined in the solid (without acid digestion) samples of ashed plant material, soil and fallout using spark emission spectroscopy with Pd as an internal standard. Statistical analysis of the data was performed using LSMEANS and CORR SAS procedures.

Soils of the investigated area had slightly acid to slighlty alkaline pH and organic carbon contents ranging from 0.2 to 4.8% with an average value of 1.2%. Fallout of alkaline particles and calcium from the remains of calcium containing materials used in building construction resulted in slightly higher (about 0.30 units) pH of downtown soils. Contents of organic carbon in downtown soils was also higher than in soils of suburban areas. Concentrations of Cu and Pb in fallout was in the range of 66-612 and 70-479 mg kg^{-1} respectively, and significantly exceeded the concentrations of these metals in suburban soils. In contrast, the Cr concentrations in fallout (16-90 mg kg^{-1} with an average value of 45 mg kg^{-1}) were in most cases below the Cr content of suburban soils. Soils of the densely populated or industrial districts of the town were contaminated with Cu and Pb; metals whose concentrations in fallout exceeded the geochemical background. The mean concentration of Pb and Cu in downtown soils (20.5 and 22.4 mg kg^{-1}, respectively) were significantly higher (at $P<0.05$) than the average concentrations for suburban areas (11.5 and 8.6 mg kg^{-1}

respectively). There was no significant difference in soil Cr levels between zones. Plant response to the soil pollution was moderate. The average Cu concentration of 9.1 mg kg^{-1} in the dry matter of plants collected in the downtown zone was only 1.2 mg kg^{-1} higher than that of plants from suburban areas. The mean value of Pb in plant tissue from downtown only exceeded the average concentration of plants from the suburban zone (6.5 mg kg^{-1}) by about 1.0 mg kg^{-1}. There was a high correlation between Pb and Cu concentrations in plants collected in downtown ($r=0.91$, $P<0.0001$) indicating that both metals come from the same source of pollution. This correlation was not evident in the suburban areas. Copper uptake by plants was highly affected by soil organic carbon. A concentration ratio defined as the ratio of Cu concentration in plant matter to the concentration in soil was negatively correlated with soil organic carbon ($r=-0.73$, $P<0.001$). It appears that human activity leading to a contamination of urban soils with Pb and Cu is associated with changes of soil properties which depressed plant uptake of metals from the soil. Probably air-born contaminants exist in a chemical form not easy available to plants. Chromium concentration in the soil was not affected by human activity and, accordingly, there was no difference in plant content of this metal between suburban and downtown soils. The petrographic differentiation of the city area had pronounced effect on Cr concentration in soils. Soils developed from a loess had higher content of Cr (54 mg kg^{-1}) than those developed from sands and loams (41 mg kg^{-1}). Plants showed a very clear response to the difference in soil parent material; the average Cr concentration in plants from the loess area was almost double (0.67 mg kg^{-1}) that of plants collected from sandy and loamy soils.

EFFECTS OF ABANDONED MERCURY MINES ON TERRESTRIAL AND AQUATIC ECOSYSTEMS

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Cinnabar deposits in the Mt Amiata district (central Italy) were first mined by the ancient Etruscans and since their rediscovery in 1849, the total mercury output (from 42 mines and 4 distillation plants) has been more than 3.5 million flasks (34.5 kg each). Owing to the gradual decline in the demand for and price of mercury, production ceased in 1980. Metal contaminated wastes are still a problem around abandoned smelting plants and attention has recently been paid to the reclamation of mining areas and their restoration.

The aim of this communication is to highlight the results of studies performed in the last decade in the Mt Amiata mining district for the purpose of identifying mercury "hot spots" and reinstating terrestrial and aquatic environments.

Although mercury occurs mainly as cinnabar (i.e. a sulfide relatively stable to oxidation and with a low solubility) in the soil and mine wastes, in the air a few centimeters above them, concentrations of gaseous mercury can reach values one or two orders of magnitude higher than in non-mineralized areas.

Mercury vaporization depends on air temperature, barometric pressure, wind, vegetation cover, and soil features, including its mercury content, pH, redox conditions, moisture, and humus content. Concentrations of atmospheric mercury, above contaminated ground show marked spatial and temporal (diurnal and seasonal) fluctuations and to obtain significant data on the degassing rate of a site, measurements must be taken daily for a year. In the Mt Amiata mining district, some species of epiphytic foliose lichens have been found to be very suitable biomonitors of gaseous mercury. Maps of mercury distribution in lichens enabled the main emission sources to be identified and subsequently monitored to verify environmental recovery.

Streams and soils in the mining district are heavily polluted. For example, mercury droplets are common in the bed of a stream running under an abandoned mercury smelting plant. The distribution and transport of the metal through biotic and abiotic compartments of the aquatic ecosystem has been widely surveyed and concentrations of soluble inorganic forms of mercury in the stream water were rather low. The metal was found to accumulate above all in sediments, suspended particulates and in biota. Notwithstanding their high mercury content, freshwater organisms did not show evident toxic effects.

Mosses, algae and the digestive gland of benthic invertebrates showed the highest bioaccumulation. The very high concentrations found in the gut, kidney and liver of fish and amphibians indicate intense uptake and elimination of inorganic mercury; concentrations of methylmercury in muscle tissue were 5-10 times lower. Although the feeding habits and size of fish were the main factors influencing mercury accumulation, the movement pattern of each species was also relevant.

The measures taken to prevent mercury emission and to reinstate the terrestrial and aquatic environments are also discussed.

ECOLOGICAL MONITORING OF SOIL INDICED UNDER CONTROL OVER THE CONTAMINATION OF SOILS WITH HEAVY METALS AND ORGANIC POLLUTANTS (METHODOLOGY, PERIODICAL OBSERVATION AND METROLOGICAL PROVISION)

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Efficient ecological monitoring dictates the necessity to choose the most sensitive indices of controlled soils, methods to specify them and the system of processing the data obtained for further application in actual practice.

Depending on tasks to be solved soil monitoring should be distinguished as operative and predicted one. The first is substantiated by real facts of chemical pollution of soils with heavy metals, oil products and pesticides. Predicted monitoring is based upon preliminary elaboration of forecasting models in conformity to technical projects to be realized. Thus, every trend of monitoring gets more effective because of a set of specific indices in polluted soils and employment of definite methods to determine them.

Operative monitoring is always made in polluted areas with the view to receive the complete information about the state of soils, the conditions for living organisms, soil rehabilitation and rational management. A priority index is the level of chemical pollutants concentration in soils, which may cause a toxic-ecological situation. In case of operative monitoring it is feasible to construct the data of soil chemical pollution, to assess its level and to recommend the land use option depending on different soil types, landscapes and anthropogenic effects.

The level of the chemical pollutants content in soils under control is usually estimated by adopted normative values. In most cases it seems to be approximated. For this reason it has been very acute to elaborate approaches and methods of ecological normalization of the soil chemical state based upon the existing ideas about ecosystems taking into complete account peculiar behavior of chemical pollutants in different soils and landscapes.

Predicted monitoring is aimed to receive information about possible unfavorable toxic-ecological situation which may appear in areas potentially dangerous in terms of chemical pollution. Such monitoring is affected as local and regional levels. It enables to control the territories which are subject to chemical pollution around industrial enterprises as well as in agricultural zones, where intensive agrotechnical measures, reclamation and irrigation may cause the soil cover degradation manifested through the humus loss, soil salinization, acidification, alcalinization, water logging, erosion and pollution.

Controlled indices of predicted monitoring are mainly specified and differentiated taking into consideration kinds of soil pollutants, soil types and systems of farming.

Efficient predicted monitoring involves the elaboration of methods for earlier diagnostics of unfavorable changes in the soil properties caused by chemical pollution and the ways to differentiate the soil according to their resistance to various pollutants.

The fundamental base of possible forecasting is a comprehensive theoretical analyses of mechanisms responsible for changes in soil indices under control, experiments and field observations of changes in the content and forms of soil-pollution substances in space and time, their direction and rate of mobility, transformation or decomposition in soils.

Every trend of monitoring needs a certain restricted set of indices in controlled soils, dominated by integral characteristics, thus providing for monitoring of soils at different levels (pedon, polypedon, soil aerial, soil cover).

The methods to specify indices in controlled soils should meet requirements to open vast possibilities for realizing the program of monitoring. Spectral, ionometric, colorimetric, voltamperemetric, chromatographic techniques are considered as express-methods to obtain reliable and precise data. Completed, dehumified and polluted soils over the vast areas under control may be received by means of remote soil monitoring to show quantitative dependence between the coefficients of spectral reflectivity and physical, chemical soil properties in natural and technogenic-transformed landscapes.

In case of predicted monitoring the requirements to periodical observation of soils depend on intensive inflows of soil-polluting substances and may be empirically established at the first stage of monitoring.

Metrical provision of soil-agrochemical monitoring should include elaborating a series of standardized soil samples, differentiated according to their composition and kinds of chemical pollution as well as unified methods of soil analyses and laboratory tests thancs national and international certification. It also requires the elaboration of methods to study variations of the controlled soil indices in space and time in order to identify more clearly essential changes in the state of polluted soils.

It is noteworthy to emphasize that soil chemical monitoring most effective only due to unification of methods to specify soil indices under control over the chemical pollution, national and international standardization, due to the use of similar measuring units, united requirements to calculation, presentation and interpretation of data obtained in the course of periodical soil observation, unified gradation of soil according to the degree of their changes caused by anthropogenic effects and chemical pollution in particular.

COMPARATIVE STUDY OF CADMIUM ACCUMULATION BY TWO SPECIES OF EARTHWORMS

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The aim of present investigation is to assess the temporal dynamics of cadmium accumulation from contaminated soil by two species of earthworms in reference to their ecology in experimental condition. The earthworms *Lumbricus r.rubellus* and *Octolasion lacteum* were selected to study. The earthworm *Lumbricus r.rubellus* is known to dwell largely in forest litter and upper soil layer. The earthworm *Octolasion lacteum* is soil dweller. Earthworms of these species are widespread in Dnieper region, therefore they were chosen to test.

Animals for experiment were collected from relatively pure biotopes.

The animals used for experiments were adult, with a well developed clitellum. Earthworms were put into the vessels with different degree of soil contamination (20, 40, 80 mg Cd/kg dry soil). Soil was treated by toxicant in the form of chloride (Cd Cl2.2H2O). Analyses to estimate cadmium concentration were performed after 2, 5, 10, 20 days of experiment. The control worms of species *Lumbricus r.rubellus* and *Octolasion lacteum*, kept in untreated soil, contained 0.24 and 0.59 mg Cd/kg dry weight respectively.

Cadmium accumulation was found to be depended on the degree of soil contamination and period of exposure.

As a result of investigation, it was stated that there is distinction of pollutant accumulation intensity by these earthworms. Concentration coefficient for *Lumbricus r.rubellus* is more than 1.00 (1.74-3.30). Whereas this parameter for *Octolasion lacteum* was revealed as being less than 1.00 (0.79-0.83). It may be stated that ecological specialisation is a most important factor affecting on the cadmium accumulation.

THE SOIL COMBINED POLLUTION OF Cd, Pb, Cu, Zn, As AND THEIR PREVENTION

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Cd, Pb, Cu, Zn, As are a group of polluting elements which coexist in the wastewater of Pb-Zn mining and smelting operation. They lead to combined pollution to the soil environment through wastewater irrigation and sludge application.

Based on pot and field experiments in Shenyang Ecological Station, this research focuses on the combined ecological effects of these five elements in dry (poplar, pine, soybean, corn and alfalfa) and paddy field (rice). The effects of modifying soil property (such as adding lime, applying Ca, Mg, P fertilizer, adding humic acid) is also included.

The results show that there is difference between the biogeochemical behavior of As and the other four elements (in which Cd is considered typical). In dry field (soybean), content of As in seed decreased by 2.5-7.8 times while content of Cd in seed increased by 1.8-4.7 times compared with the contents of As and Cd in rice seed. Adding Ca, Mg, P fertilizer inhibited the activity of heavy metals but increased the activity of As in paddy field. It is difficult to prevent the combined pollution of Cd, Pb, Cu, Zn and As.

COPPER OR ZINC RICH SEWAGE SLUDGE APPLICATIONS TO AN ERODED SOIL: II. EFFECT ON THE PHYSICAL PROPERTIES

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INTRODUCTION. Possible effects of sewage sludge application to soils concern soil physical conditions. Properties such as bulk density, porosity, moisture characteristic, hydraulic conductivity and aggregation may be affected, in a positive or negative way, by sludge application. Changes in physical conditions were looked for in an eroded silt loam where sewage sludges were added (2). The sludges came from two origins (Évora and Elvas) and were applied at five different rates (0, 10, 20, 40 and 80 t ha^{-1}). Soil physical properties, before and after a three year field trial were compared.

MATERIAL AND METHODS. Three soil profiles were sampled before and after the trial. Disturbed and core samples were taken for all treatments and also at a site subject to tillage, like the treatments, but where no sludge was applied (zero treatment). Texture, hydraulic conductivity, soil moisture characteristic, bulk density, total porosity and aggregate instability were determined in disturbed samples. Bulk density, moisture characteristic curve, porosity kinetic parameters (3), saturated and unsaturated hydraulic conductivity were determined in core samples (1).

RESULTS AND DISCUSSION. The comparison of results has shown that, as far as bulk density, porosity, maximum water holding capacity and hydraulic conductivity are concerned, differences found between the initial (Table 1) and final states (before and after the trial) were not due to sludge application but to tillage.

Table 1. Physical characteristics of core samples (initial)

Profile		1		2	3	Mean	Standard
Prof. (cm)		0-10	12-22	0-10	0-10	value	deviation
Bulk density		1.47	1.47	1.47	1.54	1.488	0.0350
Total porosity (cm^3cm^{-3})		0.488	0.488	0.503	0.459	0.4845	0.01841
Max. water hold. cap. (gg^{-1})		0.332	0.332	0.342	0.298	0.3260	0.01925
Water content	10 kPa	0.211	0.211	0.211	0.202	0.2088	0.00450
(gg^{-1})	50 kPa	0.170	0.170	0.165	0.163	0.1670	0.00356
at the suctions	100kPa	0.152	0.152	0.146	0.146	0.1490	0.00346
	1500 kPa	0.079	0.079	0.071	0.071	0.0750	0.00462
Hydraulic conduct. (cm h^{-1})		5.1	2.3	3.4	6.0	4.20	1.663

One of the possible effects of sludge applications concerns aggregation and aggregation instability. Changes in aggregation conditions are revealed by comparing porosity kinetic parameters (4). They showed that, in general, sludge application tended to decrease type 4 porosity and to decrease type 3 porosity, that is, to enlarge some of the tighter pores, meaning microaggregate development. In the zone of larger pores, only Évora sludge seemed able to increase type 1 porosity at the expenses of type 2 porosity, meaning macroaggregate development. Changes in aggregate instability are shown in Figs 1 to 3. The values for macroaggregates are very close in all determinations, denoting no effect on the low aggregation instability. For microaggregates a slight instability increase with sewage sludge

application is noticed which corresponds to a classification change from "very stable" to "stable" (clay) keeping unchanged the classification "mediantly stable" (silt + clay).

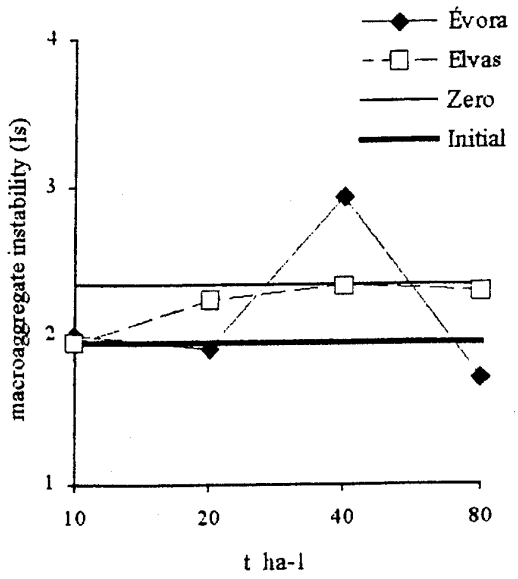

Fig.1. Mean values of macroaggregate instability (I_s) for each treatment

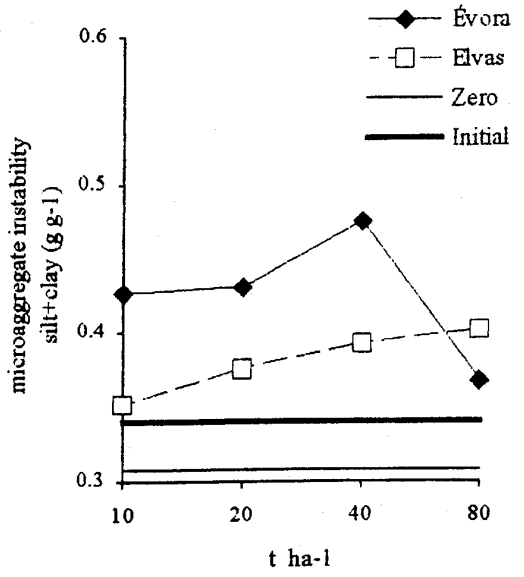

Fig.3. Mean values of microaggregate instability (silt+clay) for each treatment

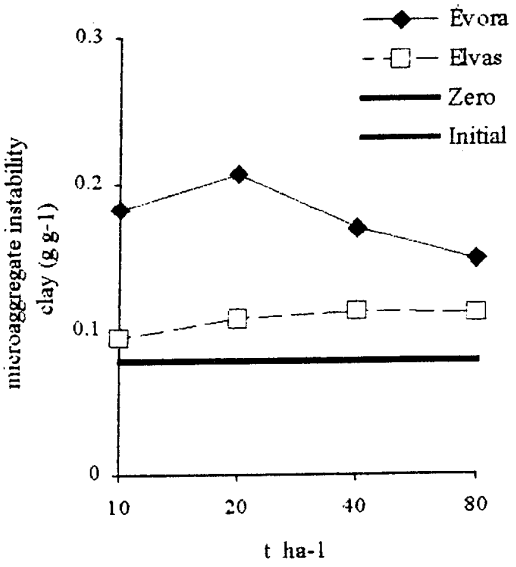

Fig.2. Mean values of microaggregate instability (clay) for each treatment

CONCLUSIONS

1 Sewage sludge application did not change soil physical properties such as bulk density, porosity, moisture characteristic and hydraulic conductivity.

2. The sludges from both origins (Évora and Elvas) had a positive effect on the relative enlargement of microporosity, meaning microaggregation developement, but only Évora sludge had a similar effect on macroporodity, meaning macroaggregate development.

3. Sewage sludge application did not change macroaggregate stability but had a negative effect on microaggregate stability: the new microaggregates will be unstable.

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EVALUATION DE L'ECOTOXICITE DES BOUES DE DRAGAGE PAR DES TESTS BIOLOGIQUES SUR VEGETAUX

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L'évaluation de l'écotoxicité des sédiments fait principalement appel à des méthodes *in situ*. Les méthodes *in situ* consistent à observer le niveau de perturbation de populations d'organismes vivant au contact des sédiments. Elles permettent d'estimer l'impact des polluants sur les écosystèmes aquatiques. Lors de l'opération de dragage, les sédiments subissent des transformations chimiques importantes. Le passage vers des conditions aérobies entraîne des modifications importantes des équilibres biologiques et chimiques dans les boues. Ces transformations peuvent certes neutraliser les effets des polluants, mais aussi, à l'inverse, faciliter leur dispersion et exalter leur toxicité. Dès lors, le choix d'une solution finale de dépôt ou de valorisation des produits de dragage doit tenir compte de leur écotoxicité.

En région wallonne, tous les produits de dragage proviennent des voies d'eau intérieures. La volume dragué annuellement varie de 600.000 à 1.000.000 m^3. Dans la majorité des cas, la solution finale est le dépôt terrestre en bassin de décantation. Notre laboratoire étudie depuis trois ans la qualité des boues de dragage et les possibilités de valorisation. En particulier, nous avons mis en évidence qu'une fraction importante de ces produits est potentiellement recyclable comme terre de culture « senso lato » (aménagement d'espaces de loisir, réhabilitation de sites industriel, recouvrement de décharges, ...). Notre étude est également basée sur des tests biologiques sur végétaux. Les plantes constituent la voie d'entrée majeure des polluants dans les chaînes et, à ce titre, sont des réactifs biologiques idéaux en fonction de l'objectif de classement poursuivi.

Le premier réactif biologique utilisé est l'algue verte unicellulaire *Scenedesmus subspicatus*. Ce test est défini par diverses normes internationales. En particulier, le laboratoire de Biologie végétale se base sur la norme ISO 8692. Notre étude a révélé qu'il constitue un excellent outil de « screening ». Il a l'inconvénient de nécessiter soit une lixiviation, soit l'extraction de l'eau interstitielle ce qui constitue une source de variations parfois non négligeable.

Le classement se base avant tout sur des tests sur végétaux supérieurs. Nous avons adapté la norma AFNOR x-31-202 au cas particulier des boues de dragage. Nous mesurons le taux de germination, la vitesse de croissance et la productivité d'une ou plusieurs plantes tests cultivées sur la produits de dragage purs ou dilués par du sable. Toutes les conditions du milieu sont contrôlées. Environ 15 espèces sont utilisées telles que *Lolium multiflorum*, *Medicago sativa*, *Hordeum vulgare*, *Nicotina tabacum*, *Thlaspi caerulescens* subsp. *calaminare*, etc... Ces tests nous permettent également de calculer des taux de transfert sol - plante.

Nos résultats permettent de qualifier les boues extraites de certains biefs, et de préciser leur vocation. Celle-ci est soit le recyclage en agriculture ou foresterie, soit la mise en décharge, soit encore d'autres types de recyclage ou d'élimination. Une ségrégation basée sur la dimension des particules présentes dans les sédiments fait apparaître que la fraction de dimension très réduite est celle qui présente la plus grande toxicité.

TENEURS FOLIAIRES EN METAUX LOURDS DE PEUPLIER, FRÊNE, ROBINIER EN PRESENCE DE BOUES D'EPURATION: VALEUR COMME BIOINDICATEURS DE POLLUTION

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La réponse des espèces forestières à une pollution du sol par des métaux lourds est peu connue. Cependant certaines espèces qui s'installent spontanément dans les terrains abandonnés pourraient servir de bioindicateurs. Trois essais, réalisés par Le Tacon et Garbaye (1978, 1988) pour tester l'effet bénégique sur la croissance de certaines espèces de boues de station d'épuration urbaine, offraient l'opportunité d'acquérir quelques connaissances sur la réaction de ces espèces aux métaux lourds et sur la teneur de leurs feuilles en métaux lourds totaux.

Dans la première expérience (Le Tacon *et al.* 1979, Jacquin et Sulce, 1992), une seule dose de boues, très élevée (240 tonnes.ha^{-1} en matière sèche), a été employée en comparaison avec un témoin sur du Robinier (*Robinia pseudoacacia*).

Dans la seconde, sur peuplier (*Populus trichocarpa*), une seule dose de 61 tonnes.ha^{-1} a été utilisée.

Dans la troisième, sur Frêne (*Fraxinus excelsior*), des doses croissantes de boues (24, 61, 146 tonnes.ha-1 de matière sèche) ont été comparées à un témoin d'une part et à un e fertilisation minérale (2 fois 75 kg/ha^{-1} de N, 180 kg.ha^{-1} de P_2O_5 et 100 kg.ha^{-1} de K_2O) d'autre part.

La composition des boues, relativement à la matière sèche, est la suivante (Le Tacon *et al.*, 1988):

C: 15,8%	Ca: 13,5%	Mn: 387 mg.kg^{-1}	Ni: 70 mg/kg^{-1}
N: 1,54%	Mg: 0,4%	Cu: 280 mg.kg^{-1}	Cd: 33 mg.kg^{-1}
P: 1,04%	Fe: 17040 mg.kg^{-1}	Pb: 767 mg.kg^{-1}	
K: 0,28%	Zn: 1946 mg.kg^{-1}	Cr: 133 mg.kg^{-1}	

Les sols forestiers sur lesquels se sont déroulées ces expériences sont des sols lessivés à pseudogley sur limon et argile du lias, à mull mésotrophe, de bonne fertilité chimique: 0,24 à 0,42 p. mille de P_2O_5 Duchaufour, 2,9 à 5,6 c.mol.+.kg^{-1}, de Ca échangeable, 0,19 à 0,33 c.mol.+.kg^{-1} de K, C/N de 16,6 et pH de 4,9 à 5,6.

A 6 ans, en 1986, la dose moyenne de 61 tonnes .ha^{-1} s'étiat révélée très positive sur la croissance du Frêne et du Peuplier, tandis que, sur le Frêne, la dose de 146 tonnes aviat tendance à être dépressive. A 13 and (1993), l'effet sur la croissance étiat encore très visible et aucun symptôme d'intoxication ne se manifestait. Dans l'essai sur Robinier, l'effet sur la croissance aviat été également très bon mais, en présence de boues, le feuillage était, en 1993, plus terne que celui des témoins.

Des prélèvements foliaires ont été effectués en août 1993 dans ces différents essais (10 arbres par traitement dans les essais sur Frêne et Peuplier, 5 arbres par traitement dans celui sur Robinier) et les éléments lourds totaux analysés (sans lavage préalable des feuilles).

Les résultats sont donnés dans le tableau 1 ci-après.

Les coefficients de variation entre arbres dans un même traitement sont très élevés: de l'ordre de 30% pour Cd, 25% pour Cr, Cu et Zn, 50 à 90% pour Ni, 20 pour Pb chez le Frêne, mais 100% pour ce même métal chez le Peuplier et le Robinier.

Aucune différence n'est significative; on s'approche du seuil de signification à 5% pour le chrome et le cuivre chez le Peuplier.

Tableau 1	Cd	Cr	Cu	Ni	Pb	Zn
			mg.kg^{-1}			
Robinier *témoin*	0.012	0.109	9.37	1.52	14.7	25.1
avec boues	0.035	0.143	6.77	0.93	10.5	22.7
Peuplier *témoin*	0.47	0.15	4.80	0.73	6.21	189.3
avec boues	0.62	0.22	5.86	0.71	13.27	210.5
Frêne *témoin*	0.019	0.13	4.01	8.57	0.80	16.9
avec boues 24t	0.012	0.12	3.71	3.51	0.52	18.2
61t	0.015	0.16	5.00	2.40	0.64	21.4
146t	0.013	0.13	4.88	3.47	0.60	20.66
fert.min.	0.018	0.13	4.48	2.71	0.66	18.3

Un rapport récent de Jacquin et Sulce (1992) au ministère de l'Environnement donne, pour l'essai sur Robinier (340 t de boues.ha^{-1}), les teneurs actuelles du sol en métaux totaux et extractibles à l'EDTA et au $CaCl_2$:

Tableau 2	Cd (mg.kg^{-1})			Cu (mg.kg^{-1})		
(Jacquin et Sulce)	Total	EDTA	$CaCl_2$	Total	EDTA	$CaCl_2$
Témoin 0-20	0.13	0.11	0.05	15.7	2.3	0.04
20-40	0.07	0.05	0.02	14.1	2.1	0.07
Avec boues 0-9	7.80	2.24	0.027	236.0	67.8	0.29
20-40	0.07	0.02	0.007	15.6	2.0	0.07
	Ni (mg.kg^{-1})			**Pb (mg.kg^{-1})**		
	Total	EDTA	$CaCl_2$	Total	EDTA	$CaCl_2$
Témoin 0-20	33.2	1.55	0.80	44.5	10.4	0.08
20-40	35.7	1.23	0.93	44.9	8.8	0.08
Avec boues 0-9	33.7	0.90	0.04	462.0	63.0	0.08
20-40	34.8	0.71	0.44	36.4	7.6	0.09
	Zn (mg.kg^{-1})					
	Total	EDTA	$CaCl_2$			
Témoin 0-20	0.70	4.0	2.49			
20-40	74.0	2.3	1.39			
Avec boues 0-9	1534.0	238.0	0.36			
20-40	78.0	1.9	0.75			

Malgré le manque de variation constaté entre arbres avec boues et arbres témoins, on peut tirer de ces investigations quelques connaissances utiles :

- l'absence de toxicité des métaux lourds étudiés pour les valeurs foliaires trouvées (sauf peut-être le cadmium pour le Robinier) ;
- l'absence de valeur indicatrice de ces espèces forestières pour les niveaux ci-dessus de teneur du sol en élements totaux ou extractibles ;

- la très grande différence entre espèces :
  - le Peuplier apparaît comme un fort accumulateur de zinc (déjà remarqué sur *Populus tremula* dans le centre de la France) et de cadmium ;
  - le Frêne extrait peu de plomb du sol mais accumule activement le nickel.

DISTRIBUTION OF Cd and Zn IN ANNUAL XYLEM RINGS OF YOUNG NORWAY SPRUCE TREES GROWN ON CONTAMINATED SOIL

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Uptake and incorporation of Cd and Zn into annual xylem rings of Norway spruce plants were investigated under controlled conditions. In a pot culture experiment 4-year-old plants of *Picea abies* (L.) Karst. were planted on contaminated soil. Five treatment groups were prepared: Cd - 30 and 80 µmol/kg soil DW; Zn - 60 and 770 µmol/kg soil DW and an uncontaminated control (soil extracts with 1 M ammonium acetate). After one vegetation period basal stem segments of the plants were harvested. The stem xylem was divided into single annual growth increments. Concentrations of Cd and Zn in xylem rings were analyzed by atomic absorption spectrophotometry.

Lowest concentrations (expressed as µmol/kg wood DW) of Cd and Zn were generally found in the outermost annual xylem rings, which had been formed during the growth period on contaminated soil. Concentrations increased steadily towards the stem center.

Calculated total amounts (nmol) of Cd and Zn in the xylem rings showed a reversed pattern with highest amounts in the outer xylem rings.

The total wood dry matter (g) of the annual zylem rings increased from the stem center towards the outer annual rings. This increase was more pronounced than the concurrent increase in amounts of Cd or Zn. Therefore, concentrations of these elements tended to decrease in the younger rings.

The results show, that Cd and Zn were not exclusively incorporated into those growth increments which were formed during the contamination period. The observed distributions are probably caused by the fact that all 5 xylem rings of the trees were conducting water. Therefore, the incorporation of trace elements seems to be a function of the water transport capacity of each annual growth ring and of the number of binding sites for Cd^{2+} and Zn^{2+} ions in xylem vessel walls.

DISTRIBUTION OF ATMOSPHERIC ^{137}Cs IN SWISS FOREST SOILS ORIGINATING FROM THE CHORNOBYL NUCLEAR REACTOR ACCIDENT

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After the Chornobyl nuclear reactor accident on 26. 4. 1986 a large amount of radioactive material was deposited over western Europe. Based on measurements with permanently installed and mobile equipment, authorities drew maps showing the distribution of radioactive fallout. Because the radioactivity of soils is of minor importance for dose assessment, soil samples were only collected locally and seldomly at forested sites. To construct soil contamination maps the only data available were the measurements of radioactive fallout at selected sites and the available meteorological data. The purpose of this study was to verify the extent of soil contamination predicted from earlier measurements of radioactive fallout.

Within the framework of the Swiss National Forest Inventory, carried out between 1982 and 1986 by the Swiss Federal Institute for Forest, Snow and Landscape Research in Birmensdorf, top soil samples were collected in a 1x1 km grid over the entire forested area of Switzerland. From this period of time more than 11'000 soil samples are stored in Birmensdorf. In 1993 a second soils inventory was performed on a 8x8 km grid. Soil samples were collected from 170 profiles by soil horizons as well as by fixed depth. The activity of radioactive cesium in the 170 top soil samples was measured using gamma-ray spectrometry at the Paul-Scherrer-Institute. Results of both pre- and post-Chornobyl sampling campaigns were compared for selected regions.

In most post-Chornobyl samples, cesium-137 could still be detected. In heavily contaminated areas cesium-134 was still present as well. Based on the known ratio of $^{137}Cs/^{134}Cs$ from the Chornobyl accident, the time between deposition and sampling together with the half-life of the two nuclides, it was possible to distinguish between the activity originating from the Chornobyl accident and the bomb fall out of the 1960s.

The pattern of radioactivity in forest soil in Switzerland coincides well with the corresponding maps which were created immediately after the accident. The lowest values (0-100 Bq/kg) were found in the Swiss plateau, in the Rhone valley and in the Canton of Grisons. At the north rim of the Jura mountains and in the region southwest of the Lake of Constance the values ranged between 100 and 500 Bq/kg. Contamination is most severe in the southern part of Switzerland with values exceeding 1000 Bq/kg.

Seven years after deposition almost all the radioactive cesium is still located in the top 5 cm of the investigated soils. These findings should be useful for radioecological modeling. The mechanisms leading to cesium retention in the top soil horizons as well as the pathway of the radioactive material to the biosphere require further detailed study.

AN APPROACH TO DETERMINE SOIL COPPER UPPER CRITICAL LEVEL FOR PLANT GROWTH PER AGROECOLOGICAL UNITS IN CHILE[1]

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Chilean economic life is ruled in a great extent by mining -mainly copper- activities. Despite the impressive achieves in other production fields in the last 20 years, still 45 to 50% of the external revenue is due to copper. Ores are mainly located in the northern half of the country inducing a high environmental degradation risk -due to mining discharges- from the I to the VI regions, including the Metropolitan Region (M.R.) of Santiago.

Parallely, very fertile agricultural areas, represented by alluvial valleys, are located from the III to the VI regions, being natural receivers of mining residues. At this part of the country, sustainable development demands an environmentally friendly mining and an armonic coexistence with agriculture. Within this framework, maximum copper allowances in alluvial soils for plant growth should be among the most relevant goals to be defined. As valleys differ in basic features, soil buffer capacity among them, guidelines including toxic threshold values should recognize these differences. Therefore, a realistic sustainable development may be achieved only if soil copper critical loads are defined per agroecological units -e.g. valleys- rather than relying on constant values for the whole country.

By means of pot experiments, under greenhouse conditions at La Platina Regional Research Centre and growing *Triticum* aestivum and *Medicago sativa* plants in artificially copper-fortified top layer (0-20 cm deep) of representative soils from the Maipo-Mapocho rivers valley (M.R.), where the capital city and about 40% of the total population are located, and the Cachapoal river valley (VI Region), southern neighbour of the Metropolitan Region and where cupric soils have been detected, the main objectives of this study were to determine the copper toxic threshold of top soils and to approach a soil copper critical value per agroecological unit.

Production of *Triticum* grain was more copper sensitive than *Medicago* aerial biomass, difference reflected by the Cu-UCL values. It was noted that two soils of similar characteristics showed a reverse behavioru: Urraca (Maipo-Mapocho valley) and San Vicente soils (Cachapoal valley), which share a lacustrine origin, black color dominantly, higher organic matter contents and are mainly devoted to Allium crops. *Medicago* aerial biomass production conducted to more reliable responses than *Triticum* grain production because of a significantly lower experimental variability.

Taking *Triticum* plants into account, there was one soil (Urraca soil, Maipo-Mapocho valley) where no significant relation between grain production and copper content in soil was detected. Cuadratic regression analysis was performed for the soils with significant responses; coefficients values (R^2) ranged from 0.76 to 0.90.

For *Medicago* plants, aerial biomass production from four soils of the Maipo-Mapocho valley (Agua del Gato, Codigua, Maipo and Santiago) was not related to soil copper content.

[1]Study sponsored by the Interamerican Bank of Development (Banco Interamericano de Desarrollo

For soils with significant responses, cuadratic regression analyses were performed and high coefficients were obtained, meaning that the growth of *Medicago* plants followed this function very closely. Regression coefficient values (R^2) remained over 0.90, except for the Cachapoal and Lampa soils (0.51 and 0.69, respectively).

Calculated from the adjusted cuadratic regression curves, the Cu-UCL values (as additional total copper, were:

Soil	Origin	Total Cu content	Cu-UCL mg kg^{-1} Triticum	Medicago
a) Maipo-Mapocho valley (M.R.):				
Agua del Gato	lacustrine, fine texture	87	128	>1,500
Codigua	recent alluvial, loam study	50	51	>1,500
Lampa	recent alluvial, loamy	107	81	170
Maipo	alluvial, loamy	51	42	>1,500
Santiago	alluvial, loamy	144	66	>1,500
Urraca	lacustrine, fine texture	67	>1,500	149
b) Cachapoal valley (VI Region):				
Cachapoal	alluvial, loamy	399	14	43
Casas de Carén	recent alluvial, sandy loamy	100	44	61
O'Higgins	alluvial, loamy	313	174	305
Rancagua	alluvial, loamy	140	41	71
San Vicente	lacustrine, fine texture	109	625	406

It was evident that the Maipo-Mapocho valley top soils own a higher copper buffer capacity-leading to higher UCL values than the Cachapoal valley top soils. Compared with the last ones, the former soils present higher pH (about 0.5 plus) and E.C. values. An approach to produce a specific toxic threshold per valley may be the use of the lowest experimental threshold value, corresponding in both cases to values associated to *Triticum* plants: 42 and 14 mg additional Cu kg^{-1} for the Maipo-Mapocho valley and the Cachapoal valley, respectively, if *Triticum* plants are the indicators, and 149 and 43 mg additional Cu kg^{1} for the Maipo-Mapocho and the Cachapoal valleys, respectively, if *Medicago* plants are the indicators.

DIFFERENCES IN BEHAVIOUR OF TRACE ELEMENTS IN FOREST SOILS AMENDED WITH HEAVY METAL CONTAMINATED REST PRODUCTS

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The pool of base cations in many Swedish forest soils is declining very rapidly. This is mainly the result of two factors: i) an increased leakage rate connected to the general acidification of forest soils and ii) a net output of nutrients by the removal of biomass in harvest.

The forest industry generates a variety of rest products, e.g. wood ash and green liqour dregs. Since they have a very alkaline reaction and contain a significant part of the nutrients taken out of the forest ecosystem in harvest, they might be suitable for liming and vitalization of forest soils.

The aim of this study was to investigate the behaviour of nutrients and heavy metals in soils treated with wood ash, green liqour dregs and municipal sewage sludge, either alone or in appropriate mixtures, in relation to plant uptake of nutrients and heavy metals.

From each soil, the mor (A_{01}+A_{02}) and mineral soil (A_2+B) horizons were used. They were air dried and sieved through a 3 mm mesh. Organic matter content, pH and CEC of the soils were determined both before and after drying and sieving. The rest products were added on top of the different soils contained in 1.5 l pots and the treatments were leached with distilled water of different pH. The leachate obtained was analyzed for all nutrient elements as well as for Cd, being the heavy metal focused on in this study. Furthermore, in one part of the study, the leachate was used as a growth medium for birch seedlings. In a corresponding experiment, birch seedlings were planted directly in the soils. In both alternatives, uptake and distribution in the seedlings of selected nutrients and heavy metals were studied.

The main results obtained so far are as follows:

- The green liqour dregs decreased the pH of the soils, and mobilized humic substances. This was despite an alkaline reaction of the rest product itself. The presence of humic- and fulvic acids in the soil water should affect the solubility of many trace elements, and could also possibly affect the extent to which they are taken up by the roots, due to the formation of chelating complexes.

-The various treatments increased the uptake of Cd in plants, due to an increased mobility of this heavy metal in the soils, especially in the treatments giving a low pH.

- Wood ash amendment increased the content of Ca in the leachate, compared to controls as well as compared to treatments with green liqour dregs either alone or in combination with wood ash. In a former study, one of the authors showed that Ca inhibits the uptake of Cd in birch, both as a consequence of competition and a rise in pH.

INFLUENCE OF METAL CONTAMINATED SEWAGE BIOSOLID APPLICATION ON C AND N MINERALISATION AND MICROBIAL ENZYME ACTIVITY IN A FIELD SOIL

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Background

The benefits of applying biosolids from waste water treatment plants to agricultural soils in terms of organic matter inputs have long been recognised. However, as biosolids from urban treatment plants are invariably contaminated with a number of heavy metals, concern has been raised with regard to the impact of metals on the biological function of soils receiving biosolids. The impact of metals on the functioning of soil microbial populations and microbial driven processes is an area that has received significant attention (1). However, no such research has been carried out on characteristic Australian agricultural soils.

The objective of this paper is to assess the potential impact of field applications of metal contaminated biosolids on a number of key soil microbial processes, notably, carbon and nitrogen mineralisation and soil microbial enzyme activity and to determine if microbial indicators can be used to develop management strategies for the 'safe' application of biosolids to agricultural soils.

Methods

Soils were collected from a field experiment at Glenfield New South Wales. (Clay loam soil, pH $_{(1:5\ H2O)}$ 5.5). Biosolids were applied at two rates, 200 t dry weight ha^{-1} equivalent (LR) and 600 t dry weight ha^{-1} equivalent (HR) over a five year period. Lime was added to half the treatment plots in order to assess the effect of soil pH on metal behaviour (2). Soils were sampled six years after the last sludge application. C and N mineralisation potential of the treated soils were determined by incubation. Soil samples were incubated at 25°C in a gas tight jar together with a 10 ml lM NaOH Carbon dioxide trap. Samples were removed at 7, 14 and 28 days. Carbon dioxide evolved from incubated soils was determined by titrating excess NaOH in the trap with 0.1M HCl. Nitrogen mineralisation was determined as mineral - N in incubated soils, NH_4-N and NO_3-N were determined in 2M KCl soil extracts colorimetrically. Soil microbial enzyme activities, phosphatase (phosphomonoesterase and phosphodiesterase), and sulphatase were determined according to Tabatabai and Bremner (3,4).

Results

The concentrations of metals in all plots receiving biosolids was higher than controls with the exception of Ni. Metal concentrations (mg Kg^{-1} soil) determined in the HR plots were Cd 1.9, Cr 42, Cu 187, Pb 83 and Zn 273. Carbon mineralisation rates determined as CO_2 evolved from incubated soils were not significantly different ($P<0.001$) when control and treated soils were compared. However, for soils that had been limed, both control and treated soils had higher C mineralisation rates than unlimed soils. The pH of limed soils was on average one pH unit higher than the unlimed soils (pH 6.5 as compared to 5.5). The

mineralisation of N in the treated and control soils presents a very different pattern. In both the unlimed and limed soils N mineralisation rates were significantly higher in soils receiving biosolids than control soils. N mineralisation measured as NO_3-N in the HR unlimed soil was almost double the control soil. Again mineralisation rates in the soils receiving lime were significantly higher than the equivalent unlimed treatments. No significant difference in phosphomonoesterase enzyme activity was noted between soils receiving biosolids and control soils. This was the case for both unlimed and limed soils. However, phosphodiesterase activity was significantly lower (30% decline) in unlimed soils receiving biosolids when compared to controls. No difference was noted in limed soils. A similar pattern was observed when microbial suphatase activity was determined in unlimed soils. A similar pattern was observed when microbial sulphatase activity was determined in unlimed soils (40% decline in activity in soils receiving biosolids). The role of soil pH and liming on metal impacts on microbial activity and the different sensitivity of microbial enzymes to metal contamination will be discussed, as will the applicability of current guidelines for biosolid application to agricultural soils in protecting soil microbial communities.

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DISTRIBUTION OF HEAVY METALS IN TREES ON CONTAMINATED SITES IN WEST GERMANY

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1Introduction

The investigation was carried out within the project "Atmospheric and geogenic components in the Heavy Metal Budget of Forest Trees". It focused on separating pathways of metal uptake, which is possible via roots, bark, and assimilation organs. The separation of the uptake from soil and atmospheric deposition resepctively is a basic requirement for the determination of actual input rates into forest ecosystems.

A further aim was to demonstrate the role of internal transport processes and to find out the rules of metal distribution in the different tree organs. This knowledge is important for using heavy metal distribution patterns in tree-rings to reconstruct the immission history of a site.

2 Material and Methods

The methodical approach is based on the comparison of trees on strongly polluted soils respectively on sites with high atmospheric deposition. In order to eliminate effects of soil contamination, which occurs by the atmospheric deposition, trees were selected on carbonate and silicate substrates. It was assumed that the availability of heavy metals in the carbonate soils with pH values > 7 is very low and that the uptake by roots can be ignored. The hypothesis was that characteristic heavy metal distribution patterns exist, which depend on the type of pollution.

Feasible sites were found in the strongly polluted region of Stolberg (West Germany). Near the emittents the annual Cd-, Cu-, Pb-, and Zn-deposition-rates are 10-100 times higher than the background values of unpolluted forest sites. Corresponding investigations were carried out in the Southern Black Forest, where the heavy metal deposition is generally low. On these sites trees were selected on uncontaminated soils and on neighbouring old ore mine spoils with high heavy metal contents.

Further trees were selected on carbonatic soils aside of the highway A5 between Freiburg and Basel. A strong increase of Pb-deposition occurred after opening the highway 30 years ago. The aim was to find out, whether this leads to a corresponding increase of the Pb-contents in the tree rings. This is an important requirement for the usage of radial Pb-distributions in the stemwood to reconstruct the immission history.

The investigation concentrates on Cu, Zn, Cd, and Pb.

3 Results and conclusions

The level of the element contents depend on the available amount in the soil. This holds even true for lead which is predominantly accumulated on the surface of the leaves bark and roots. Substantial amounts were taken up from the soil. Therefore roots are the most important pathway of uptake. Even on sites with high deposition rates there was no indication of a substantial heavy metal uptake via bark or assimilation organs.

The distribution of heavy metals in the *roots* is characterized by an accumulation in the fine roots and the outer bark. The contents in the wood decrease with increasing age of the tissue. Obviously there is an effective discrimination or exclusion in the rhizosphere and on the root surface.

Except of lead the element contents were higher in the *bark* than in the *stem*wood and the *periderm tissue* often had lower contents than inner and outer *bast*. The radial distribution in the stemwood is differentiated individually and there are no element specific-patterns. In total 4 characteristic distribution types were identified, which can be explained by physiological, chemical and cation exchange processes.

The distribution in the *assimilation organs* was predominantly influenced by the available supply of heavy metals in the soil and the element composition in total. Only lead distribution was characterized by atmospheric deposition. 3 distribution types were identified.

Lead contents in the stemwood of an oak aside the highway were low and the radial distribution was not affected by the rapid increase of Pb-deposition. So it is not possible to reconstruct immission history by determining the lead distribution in tree rings.

LEAD AND CADMIUM DISTRIBUTION IN URBAN SOILS AND PLANTS IN THE CITY OF ROME (ITALY)

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In the last years many studies have stressed the importance of the study of the levels of heavy metals contamination in urban soils. Heating systems and motor vehicle traffic (lead from the exhaust system, cadmium from engine oils, metal release with the products of abrasion of tyres, etc.) represent the most important potential sources. It is also acclarated that these contaminants persist in the upper layer of soil horizons for long time.

People living in high traffic density cities are daily exposed to significant levels of Pb and Cd; these elements may principally enter into the human body by inhalation, producing severe health diseases that, sometimes, can become chronic.

As regards urban soil contamination, some studies have evidenced a relationship between dental caries in children and Pb concentration in soil, especially when Pb is present as easily bioavailable form. Children in fact result to be the most exposed population class; accidental intake may occur directly handling the contaminated soil or through the contamination of toys.

The aim of this study is to investigate lead and cadmium distribution in soils and plants (*Bellis perennis*; *Malva sylvestris*) in the urban soils and amenity areas of Rome.

Top soil (0-5 cm) and plant samples were collected along different streets in relation to different traffic density areas. Sampling was performed considering site morphology and its local influence on prevalent wind direction, and distances from streets. Many soil and plant samples were deliberately collected where pollution was supposed to be at the highest level: generally 1-3 m from the streets.

With the aim to verify whether the seasonal variation in the life cycle of plants induces differences on metals uptake from soil and accumulation, plants were collected in two periods of the year: autumn and spring.

Data show a significant correlation between the vehicular traffic intensity and Pb and Cd concentration levels in soils.

Very high concentrations of Pb have been found in the heavy traffic areas of the centre of the city (concentration range: 200-1,400 mg/kg d.w.). Unexpectately, Cd concentrations result to be relatively low (concentration range : 0.1-1.9 mg/kg d.w.).

As expected, lead and cadmium levels both decrease with the distance from streets; generally the highest levels were found within 3-10 m form the source; values close to the background levels were only measured at distances greater than 80-100 m from the streets.

Moreover it was found that site morphology affects Pb and Cd distribution along transects.

In some soil profiles a clear decrease with depth, towards background values, has been observed for both elements, testifying that pollution considerably increased in recent years.

Lead and Cd are readly available to plants from air and soil; so in urban environment plants can uptake high concentrations of these elements. Lead concentration in *Malva sylvestris* and *Bellis perennis* ranges from 1 to 22 mg/kg, d.w.

Cd concentrations in *Bellis perennis* (range: 0.17-1.75 mg/kg d.w.; median 0.80 mg/kg d.w.), is higher than in *Malva sylvestris* (range 0.04-0.38, median 0.12 mg/kg d.w.). The behaviour of Cd with respect these plants species might reflect differences in the ability to

adsorb the metal from soil. Besides the differencies existing between the plants morphology and in particular between the foliar systems, could explain the higher Cd levels in *Bellis perennis*.

BACKGROUND LEVELS OF MERCURY IN SOILS OF LATIUM (CENTRAL ITALY)

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Despite the potential hazard of Hg, little attention has been paid to its distribution in Italian soils. Owing to past and recent volcanism and the related hydrotermal and gaseous activity, many anomalies of Hg have been recognized in centratl and southern Italy.

To improve the knowledge about the distribution of Hg in Italian soils, a survey has been made in soils from Latium (central Italy). This area has been interested for long time by intense volcanic activity which products have covered nearly half of its territory. Besides, pyroclastic products have reached also distal areas all around the region, covering the outcrops of the dominant sedimentary rocks and becoming in many areas the new parent material for soils.

This study has been carried out analyzing total Hg content of 125 top-soil samples collected all over the region. In some particular cases we have also considered the Hg behaviour within some soil profiles.

Soil samples were dryed at 40°C and sieved at 2 mm. Dissolution were carried out in teflon bombs in a microwave digestion system with aqua regia diluited 1/2.

The Hg determinations were obtained by C.V.M.A.A.S. and all analysis procedures were previously tested introducting international standards.

Total Hg concentrations range from less than 16 to 3,000 µg/kg dw. The average for all samples is 331 µg/kg, while the median is 102 µg/kg.

The relationships between Hg in soil with O.M., C.EC., pH and clay, show the presence of two groups of samples and the Hg content seems not to be strictly related to the parent material. In fact the samples high in Hg (average 1,960 µg/kg; range 850-3,000 µg/kg) include nearly all soils of reliefs top faces, where the parent material is represented by limestones with mercury concentration less than 90 µg/kg.

The 'low Hg' samples includes both soils on sedimentary and volcanic rocks. Statistical elaboration permit a further differentiation giving an average of 131.8 µg/kg and 175 µg/kg, respectively for each substratum.

It is difficult to justify the presence of so high Hg concentrations in soils of the first group, considering only the small differencies existing between some pedological parameter as C.S.C., organic carbon and organic matter. However the profiles distribution pattern, show a clear decrease of Hg with depth (less than 90 µg/kg in the R horizon). This finding could mean that only an atmospheric source for Hg, related with the mentioned past volcanic activity, could justify the presence of this element in these soils, while the further accumulation on top horizons might be enhanced by pedogenetic processes.

THE HEAVY METAL CONTENT OF MERSEYSIDE SOILS, 1974-1994

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BACKGROUND

In 1974 the (then) newly formed Merseyside Metropolitan County commissioned a survey of the trace metal content of urban and rural areas on Merseyside (Parry *et al.*, 1981). Two hundred samples were collected from parkland, gardens, allotments, agricultural grassland and arable land. The County had a population of 1.58×10^6 and comprised an area of 650 km^2 of which approximately 260 km^2 was occupied by urban development. The industrial base of the area included port-related industries, chemical and refining processes and petrochemical complexes. The study identified areas with elevated soil levels of Cd, Cu, Pb and Zn and clearly demonstrated the value of soil survey and mapping techniques to data base development for planning purposes.

AIMS

In the 20 years since the original investigation the population of Merseyside has decreased, there have been major changes in the industrial structure of the county and legislation relating to atmospheric emissions has also been amended. There has also been increased interest in soil contamination in urban areas. The authors of this present study have obtained the data from the 1974 survey. The aim of this investigation is to compare contemporary heavy metal content of soils in one area of Merseyside with those analyzed in 1974. The results presented focus on the Wirral peninsula, a borough of the Merseyside county area.

METHODS

Fifteen sites were selected on the Wirral peninsula. All sites had been included in the 1974 survey. Soils were sampled at two depths: 0-20 cm and 20-40 cm. Soils were air dried and sieved through a 2 mm mesh. Samples were analyzed for pH and loss-on-ignition. Heavy metals were extracted with 0.5M acetic acid and 0.05M EDTA following the same methods used for the 1974 survey. All analyses were carried out in triplicate.

RESULTS

Table 1 shows the range and mean metal concentrations for 1974 and 1994.

Table 1 Metal concentrations (mg/kg) for surface soil samples, Wirral, 1974-94

Metal	**1974**		**1994**	
	Range	Mean	Range	Mean
Cd	0.04-0.49	0.16	0.1-0.5	-
Cu	8.9-346	45.1	<0.1	-
Pb	1.4-26.4	6.74	0.4-43	12.6
Zn	4.5-2730	251.1	1.0-2400	34.7

The results show a significant reduction in the copper and zinc content of Wirral soils over 20 years but an increase in the mean lead concentration. The spatial variation in results will be presented and evaluated in the context of likely metal sources. The problems of monitoring metal concentrations in urban soils will be discussed.

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COPPER OR ZINC RICH SEWAGE SLUDGE APPLICATIONS TO AN ERODED SOIL: I. ACCUMULATION OF Cu AND Zn ON SOIL AND SUBCLOVER

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INTRODUCTION - Since 1991 there are rules in Portugal which limit the use of sludge in agricultural land, according to metal concentration, but guidelines for final use of urban sewage sludge are needed for farmer's use. Portuguese rules [1] allow the application to agricultural land of 6 t ha^{-1} of sludge or more if heavy metal concentration is lower than the EU limits.

The disposal of sludge on agricultural land has beneficial effects on soil fertility and agricultural productivity, but the phytotoxicity effects and soil contamination need monitoring studies.

The aim of this paper is to report the results of application of Portuguese urban sewage sludge in a field experiment concerning crops phytotoxicity and soil contamination.

MATERIAL AND METHODS - In a field experiment two kinds of urban sewage sludge, rich in copper (Évora sludge) or zinc content (Elvas sludge), were separately applied to an Eutric Lithosol (Ie) [2], cropped with subterraneum clover, Cv. Clare and Nungaring, non-inoculated and inoculated with a Portuguese selected strain. The soil was tilled and treatments were in triplicate and randomized split split plot block design. The plots has 1x2 m. The rates of sludge applications were 0, 10, 20, 40, 80 t ha^{-1}; a mineral fertilization was done with 600 kg ha^{-1} of superphosphate 18% and 150 kg ha^{-1} of KCl 50%. Soil was limed at the rate of 2000 kg ha^{-1}. The yield of first year was lost due to dry weather in this region, but analysis of soil samples from some plots was done. In the second year, two cuts of subclover and foliar analysis were done. Yield were statistically analysed using Least Significant Difference Test (MSTAT version 4.0). Soil and sludges were initially characterized and the amount of heavy metals added to soil was calculated.

RESULTS AND DISCUSSION - The soil had pH=6.0 and low levels of O.M. (13.4 g kg^{-1}), N (1.09 g kg^{-1}), available P (18 mg kg^{-1}), available K (40 mg kg^{-1}), CEC (11.8 $cmol_c kg^{-1}$) and heavy metals (Cd= n.d.; Cr=60; Cu=16.7; Ni=27; Pb=45; Zn=52 mg kg^{-1}).

The sludges showed high concentrations of Cu in Évora sludge (Cu=790, Zn=969 mg kg^{-1} D.M.) or Zn in Elvas sludge (Zn=2025, Cu=264 mg kg^{-1} D.M.).

Treatments of 40 and 80 t ha^{-1} of Évora or Elvas sludge will add to the soil, by calculation, Cu and Zn (kg ha^{-1} y^{-1}) higher than the limits of Eu Directive [3] (Elvas sludge - 40 t ha^{-1} adds Zn=37.6 and 80 t ha^{-1} adds Cu=12.6 and Zn=75.2; Évora sludge - 40 t ha^{-1} adds Cu=20.2 and 80 t ha^{-1} adds Cu=40.3 and Zn=51.8). After the 1st year, soil analysis showed that only the treatment with 80 t ha^{-1} of Évora sludge had Cu higher (67 mg kg^{-1}) than the limits of EU Directive.

Subclover yield (Fig.1) was higher in the 1st cut than in the 2nd cut. In the 1st cut, the rates were statistically different ($P<0.01$) between PK, 40, and t ha^{-1} (a) and 0, 10, and 20 t ha^{-1}. In the 2nd cut the rates were statistically different ($P<0.01$) between PK (a) and the others treatments (b).

analysis of the 1st cut showed adequate 4Cu mg kg^{-1}) in most of the treatments and Cu slightly high (15 mg kg^{-1}) for treatment with 80 t ha^{-1} of Évora sludge; and showed low levels of Zn (12-38 mg kg^{-1}) in most treatments with exception of treatments with 40 and 80 t ha^{-1} of Elvas sludge (50-76 mg kg^{-1}). For 2nd cut foliar Cu and Zn were more elevated (Cu=12-17 mg kg^{-1} and Zn=23-82 mg kg^{-1}).

In soils poor in organic matter and heavy metals the application of sludges high Cu or Zn content was beneficial to subclover yield and subclover did not show phytotoxicity effects. Soil accumulation of copper was verified when sludge was applied.

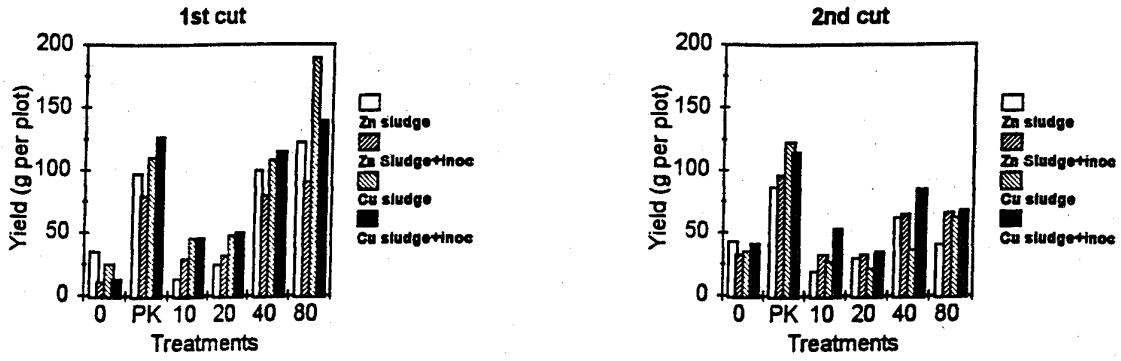

Fig 1. Yield of subclover.

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SOIL MICROBIAL BIOMASS CONTENT OF SEWAGE SLUDGE-TREATED AGRICULTURAL LAND

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The principal functions of the soil microbial biomass are the decomposition of organic matter and to provide a reservoir of labile plant nutrients. Changes in the size and activity of the soil microbial biomass may give an indication of the potential consequences for long-term soil fertility of different soil management practices. Reductions in the size of the soil microbial biomass have been reported apparently due to increasing concentrations of heavy metals in sludge-amended agricultural land, implying potentially an impairment of soil fertility. Agricultural utilisation of sludge is currently regulated to avoid phytotoxic effects on crops and to protect the human food chain from heavy metals applied to soil in sludge. To assess the extent of possible effects on the soil microbial biomass of the maximum permissible concentrations of potentially toxic metals in sludge-amended farmland in the UK, field soils with long histories of sludge and organic waste application, and with contrasting metal profiles, were sampled for the determination of microbial biomass content. The results indicated that the microbial biomass may be particularly sensitive to elevated Cd concentrations in soil. However, Cd is rarely the element limiting sludge recycling as the concentrations present in sludge have been drastically reduced due to changes in industrial practices and effective trade effluent control imposed by the Water Industry. Therefore, experimental soils used previously for assessing the potential impacts of heavy metals on soil micro-organisms, which are rich in Cd in relation to the soil limits for other important elements such as Cu and Zn, are probably unsuitable for examining the possible effects of current and future sludge application practices on soil fertility. No significant effect of Cu on microbial biomass content was detected in a mono-metallic soil treated with distillery waste and containing up to 460 mg Cu kg^{-1}. The upper critical concentration of Zn was estimated to be >500 mg Zn kg^{-1} for a coarse-textured soil with pH value in the range 4.9-6.4. The results are discussed in relation to current European soil limits for heavy metals in sludge-treated agricultural land.

EFFECTIVENESS OF *RHIZOBIUM LEGUMINOSARUM* BIOVAR *TRIFOLII* STRAINS IN SOILS POLLUTED BY INDUSTRIAL EFFLUENTS

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INTRODUCTION

Industrial activities and the disposal of waste products have resulted in the contamination of many terrestrial environments. Industrial effluents often contain considerable amounts of potentially toxic metals such as Cu, Zn, Ni, Cd, Pb and Cr. Heavy metals are known to be persistent in the cultivated layers (ref. 7) and can have ecotoxicological effects on plants and soil microorganisms.

There is an increasing evidence of adverse effects of heavy metals on soil microbial process, including the symbiotic N_2 fixation. McGrath *et al* (ref. 6), found a marked suppression of symbiotic nitrogen fixation by *Rhizobium leguminosarum* bv. *trifolii* in symbiosis with *Trifolium repens* L. from soils contaminated with metals, at Woburn, UK, as a result of sludge application more thant 20 years. Later studies in the same soil confirmed that the surviving rhizobia were ineffective at fixing N_2 with the host plant and lacked genetic diversity (ref. 2). Deleterious effects of metal contamination on survival of *Rhizobium* have also been observed (ref. 1, 3, 5 and 9).

The objective of the present experiments was to evaluate if the effectiveness of *R. leguminosarum* bv. *trifolii* population had been affected detrimentally by the presence of heavy metals in soils from an industrially polluted area.

MATERIALS AND METHODS

Sampling and analysis

Two composite soil samples were collected from arable soils (0-20 cm depth), at Estarreja, in centre of Portugal. One of them, polluted by heavy metals, was collected near an industrial effluent channel (I). The other was collected from a similar soil unpolluted (IA). No clovers were present in either field. Total concentrations of heavy metals and pH are given in Table I.

Table I. Total metal concentrations and pH in Estarreja soils.

Soil	Total metal concentration (μg g^{-1} soil)						pH
	Cd	Cu	Zn	Ni	Pb	Cr	
I (polluted)	1.50	117.0	375.0	4.00	97.5	14.5	6.17
IA (unpolluted)	0.40	29.0	75.0	6.50	41.5	13.0	6.00
EC limits*	1-3	50-140	150-300	30-75	50-300	100-150	6-7

*E.C. limits for sewage sludge treated soils.

Estimation of population size of *R. leguminosarum* bv. *trifolii*

Numbers of *R. leguminosarum* bv. *trifolii* were estimated by the most probable number (MPN) method, using *Triflium subterraneum* L. cv. Clare as host and a 10-fold dilution series (ref. 10).

Estimation of symbiotic effectiveness of *R. leguminosarum* bv. *trifolii*

Subclover cv. Clare was seeded in pots filled with each soil and maintained under controlled conditions. After 5 weeks growth, clover plants were removed from soils and the nodules carefully excised from each plant, rinsed, surface sterilized and crushed for single colony isolation. The isolates (27 from the polluted soil and 31 from the unpolluted soil) were used to inoculate subclover seedlings (six replicate per isolate), growing in Jensen's agar medium (ref. 4) in a controlled environmental growth cabinet. After 3 weeks growth, the acetylene reduction assay (ARA) was used to test for nitrogenase activity.

RESULTS AND DISCUSSION

Soil I had greater total concentrations of heavy metals than soil IA. The Zn concentration exceeded the highest E.C. limits for arable soils (Table I).

The population size of *R. leguminosarum* bv. *trifolii*, in soils I and IA was the same (Table 2) and seems not to be affected by heavy metals because no reduction on rhizobial population was detected in soil I.

The results obtained from ARA determination (Table 2), showed that the isolates from contaminated soil (I) were different from the uncontaminated soil (IA). Almost all of the isolates from IA had positive ARA, while most the isolates from soil I lost this ability. It appears that the heavy metals exerted a genetic effect on the rhizobial population rather than a quantitative effect on the population size. Although our results are in opposition to those from Obbard & Jones (ref. 8), the support the idea that heavy metals had a negative effect on the effectiveness of rhizobial population, agreeing with those from the Woburn experiment (ref. 2 and 6.).

Our results of plasmid profiles of isolates from the polluted soil showed the presence of a narrow genetic diversity among the rhizobial population, contrasting with those from the unpolluted soil.

Table 2. Numbers of *R. leguminosarum* bv. *trifolii* of Estarreja soils estimated by the most probable number and % of effective strains determined by acetylene reduction assay.

Soil	N° of *Rhizobium* g^{-1} soil	Fix^+	Fix^-
I	$2.3x10^4$	18.5	81.5
IA	$2.3x10^4$	93	7

Fix^+ presence of nitrogenase activity
Fix^- absence of nitrogenase activity

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NICKEL, CADMIUM AND MAGNESIUM CONCENTRATIONS IN XYLEM RINGS OF QUERCUS ILEX L. FROM SERPENTINE SOILS OF NORTH-EAST PORTUGAL

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Serpentine soils have high concentrations of Ni, Cr and Co, unfavourable Mg/Ca ratios and low levels of N and P. The flora of this soil is rich in endemic species.

Quercus ilex L. is a tree species that grows on serpentine soils of north-east Portugal. It shows stunted growth, when compared with *Q. ilex* from non-serpentine soils.

The aim of this work is to investigate differences in the amounts of Mg, Ca and Ni in xylem rings of *Q. ilex* from a serpentine and a non-serpentine soil. Radial distribution patterns of these elements in stem wood are also studied.

The trees were cut and xylem samples were obtained with a borer. The wood samples were digested under pressure with concentrated HNO_3 in teflon vessels at 150°C. Concentrations of Ca and Mg were determined by flame atomic absorption spectrophotometry (Perkin-Elmer 380). Concentrations of Ni were determined by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 5100).

Concentrations of Mg in xylem rings of *Q. ilex* were higher in the serpentine area, with a mean value of 83.7±7.1 µmol/g wood dry wt. In the control area the mean value of Mg was 31.8±17.2 µmol/g wood dry wt.

The amount of Ca in xylem rings was lower in *Q. ilex* from the serpentine area. The mean value was 35.0±22.4 µmol/g wood dry wt, compared with 101.1±24.4 µmol/g wood dry wt from the control area.

Mean Ni concentrations in stem wood were higher in the serpentine (70.0±48.1 nmol/g wood dry wt) than in the control area (11.3±6.7 nmol/g wood dry wt).

The radial distributions of Mg, Ca and Ni in stem wood showed higher concentrations levels in the stem centre. Concentrations of Mg decreased towards the cambium while Ca and Ni showed an increase.

EVALUATION DU RISQUE DES SOLS POLLUES POUR LES OISEAUX ET LES PETITS MAMMIFERES

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La pollution des sols a pour origine la fabrication, l'utilisation ou le rejet de produits chimiques très divers qui se dispersent ensuite dans l'environnement de façon incontrôlée. La faune sauvage est exposée à la pollution des sols soit par un contact direct avec le sol, soit par l'air, l'eau ou les végétaux. Les prédateurs courent un risque supplémentaire du fait des possibilités de bioaccumulation dans les chaînes alimentaires. La nature des problèmes dépend des situations: pratiques agricoles (utilisation de pesticides, d'engrais minéraux, de boues de STEP), présence de friches industrielles, de mines ou de sites de décharge, constituent autant de problèmes différents. Les modes et les niveaux de pollution sont très variables, depuis la pollution continue et diffuse des sols par des traces de produits jusqu'à des pollutions très intenses et localisées (accident de camion ou fuite d'un oléoduc) ; les sites pollués peuvent être très anciens, comme le voisinage de certaines mines, ou au contraire beaucoup plus récents.

La démonstration - sans équivoque - des effects du DDT et de la dioxine sur les populations d'oiseaux montre que la toxicité des polluants environnementaux est une réalité. Différentes informations sont nécessaires pour évaluer le risque qu'ils font courir à la faune sauvage : (1) la *caractérisation du niveau de pollution du milieu*, (2) la *définition des espèces et des populations à étudier* et de leur *degré d'exposition* et (3) la *caractérisation des effets* (nature des effets et relations dose-effet).

Ces informations et ces données sont générées par *différentes approches*, tests d'écotoxicité en conditions de laboratoire, études en microcosmes ou en mésocosmes, ou encore surveillance biologique de l'environnement. Par exemple, la *mortalité*, qui est de loin le critère le plus fréquemment employé pour caractériser la toxicité, est facilement caractérisée par des tests d'écotoxicité au laboratoire aboutissant à des expressions quantifiées (dose létale 50) ; cette mortalité est plus difficile à mettre en évidence sur les populations naturelles d'animaux terrestres, même en cas de fortes pollutions.

La *caractérisation du niveau de pollution du sol* est faite par des mesures *in situ* ou l'tilisation de modèles mathématiques.

Le *choix des espèces et des populations à étudier* est difficile : elle dépend de critères écologiques et socio-économiques, variables selon les situations, mais rarement objectivés. On définira les *espèces à risque* et, pour évaluer la qualité de l'environnement et le *risque écologique* pour la faune sauvage, on se basera sur des *espèces bio-indicatrices* ou des *animaux sentinelles* collectés sur le site.

L'intérêt d'un suivi d'animaux sentinelles pour l'évaluation du *risque humain* a été souvent souligné : d'une part, leur cycle de vie, moins long que celui de l'homme, permet une analyse des effets à long terme des polluants, d'autre part, l'exposition est considérée comme « réaliste » par sa durée, le degré d'exposition et la prise en compte de l'ensemble des produits présents. Le surmulot (*Rattus norvegicus*) est un bon candidat pour la surveillance des écosystèmes terrestres et l'évaluation du risque humain : les études épidémiologiques sur les rongeurs collectés *in situ* peuvent bénéficier des données toxicologiques sur les rongeurs de laboratoire.

L'exposition des animaux aux polluants varie beaucoup selon les approches envisagées : exposition aux produits purs administrés dans la nourriture ou injectés (tests de laboratoire), ou mise en contact des animaux avec des matériaux bruts provenant du site (sol pollué, par

exemple). *In situ*, on peut envisager l'utilisation d'animaux encagés sur le site ou capturer des animaux sur le site pollué. Le niveau de contamination des animaux est défini dès le départ dans les tests d'écotoxicité au laboratoire, il peut être mesuré chez les animaux collectés *in situ*, ou bien estimé par des modèles mathématiques sur la base du niveau de pollution du milieu naturel.

Différents critères peuvent être retenus pour *la caractérisation des effets toxiques*, depuis les altérations moléculaires et cellulaires jusqu'aux effets pathologiques et physiologiques observés sur les individus, et les effets écologiques sur les populations et les communautés. Les altérations moléculaires et cellulaires générées par le polluant desn l'organisme sont à l'origine des effets toxiques ultimes. Le développement de *biomarqueurs*, basés sur ces effets moléculaires et cellulaires, est une des priorités des recherches écotoxicologiques actuelles. Ces biomarqueurs constitueraient des signaux d'alarme précoces et sensibles ("*early warning systems*"), premier indice du risque de développement d'effets à long terme, avant que les dommages aux individus ou aux populations soient irréversibles.

De très nombreux paramètres biologiques, biochimiques et immunologiques, liés plus ou moins directement au mode d'action des polluants ou à la pathogenèse, peuvent servir de base pour développer et valider des biomarqueurs. Des constituants tissulaires spécifiques comme les *métallothionéines* tissulaires indiquent des pollutions métalliques. L'*inhibition* ou *l'induction de systèmes enzymatiques* sont souvent à l'origine du développement de biomarqueurs : par exemple, les *cholinestérases*, inhibées spécifiquement par les insecticides organophosphorés ou carbamates, sont de bons exemples de biomarqueurs d'exposition des oiseaux à ces produits. L'induction des *cytochromes P-450 du foie* a été très étudiée, surtout en écotoxicologie aquatique, comme biomarqueur de la contamination de l'environnement par des polluants majeurs tels que les hydrocarbures aromatiques, les PCBs ou les dioxines. Les *adduits à l'ADN* ont particulièrement retenu l'attention, car à moins d'être réparées par l'organisme, ces modifications structurales sont des évènements précurseurs de cancers.

D'autres critères peuvent servir à caractériser la toxicité, tels que les études de séquences comportementales ou le suivi de paramètres physiologiques comme le taux de croissance. L'évaluation du *succès de la reproduction* constitue un critère particulièrement important pour la survie des populations animales sauvages, mais c'est aussi un des plus difficiles à mesurer. Des *critères écologiques*, comme les fluctuations démographiques d'espèces bio-indicatrices, ou la disparition de certaines espèces sont des éléments majeurs à considérer dans l'évaluation du risque des polluants.

Les différentes approches utilisées ont des avantages et des inconvénients qui font l'objet de nombreux débats : il est souvent reproché aux tests de laboratoire d'être « réducteurs » et de manquer de « réalisme écologique », tandis que pour d'autres, la surveillance biologique n'apporterait pas de conclusions précises et serait incapable de définir des standards de pollution. En fait, aucune d'elles ne peut, à elle seule, rendre compte de tous les problèmes. Chaque situation doit être appréhendé dans son contexte particulier et l'ensemble des informations collectées et des données disponibles doit être pris en compte et intégré dans une *stratégie générale d'évaluation du risque écologique*. Le principal problème est la faiblesse des données disponibles en matière de petits mammifères et d'oiseaux.

CHICORY (*CICHORIUM INTYBUS, L.*) AS BIOINDICATOR OF HEAVY METAL CONTAMINATION

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Leafy green vegetables have a proclivity for high mineral uptake, and a tendency to accumulate nutritionally undesirable heavy metals and radionuclides (Alloway, 1990; Fergusson, 1990). Chicory have been found to be an indicator of cadmium contamination of soils or nutrient solution (Simon *et al.*, 1994). Composted municipal sewage sludge deposited into agricultural soils may contain considerable amounts of heavy metals (Adriano, 1992; Alloway, 1990; Fergusson, 1990). The objective of our study was to investigate the bioindication properties of chicory; the uptake and distribution of heavy metals (Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn) from composted sewage sludge.

Greenhouse pot experiments were conducted with chicory (*Cichorium intybus*, L, cv. Wild). The plants were grown in brown forest soil medium amended with 0%, 10%, 25%, 50% or 100% (m/m %) of municipal sewage sludge aerobically composted with wheat straw. The elemental composition of the plant, soil and composted sewage sludge samples were determined by inductively coupled argon plasma emission spectrometry (ICAP). The plant samples were digested with H_2O_2/HNO_3. To determine the soluble elemental fraction, soil and composted sewage sludge samples were shaked with Lakanen-Erviö solution (H_4EDTA in ammonium acetate buffer) before analysis.

Significantly higher amounts of Cd, Cr, Cu, Pb and Zn, and less Hg and Mn were found in the composted municipal sewage sludge than in brown forest soil. After 6 weeks of growing, the chicory shoots and roots took up Cd, Cr, Cu, Mn and Zn from the growth medium, while the Hg, Ni and Pb contents of the plants were under the detection limits. With increasing amounts of applied compost higher amounts of heavy metals were found in shoots and roots of chicory. Chicory shoots and roots indicated linearly the presence of Mn, Zn and Cd in the growth medium. Heavy metals appeared equally in shoots and roots.

Beside indication of Cd contamination (Simon *et al.*, 1994) chicory proved to be an indicator of Mn and Zn contamination, too. Chicory is a leafy green vegetable or can grow as a weed. Its worldwide endemic distribution and the ability to tolerate a broad range of climatic and soil condition makes chicory to be a potential international heavy metal contaminant indicator species.

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RESIDUAL EFFECT OF ORGANIC MATTER ON CADMIUM UPTAKE BY PLANTS AND ITS DISTRIBUTION IN SOILS

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In a greenhouse experiment, the residual effect of organic matter on Cd concentration in plants and its distribution in three soils of varying soil texture was investigated. Organic matter (sphagnum peat) was added in the first year and the residual effect was studied in the second and third year. The soils used for this study were a sand, a sandy loam and a clay loam which were amended with six levels of organic matter (0, 20, 40, 80, 160 and 320 g kg^{-1} of the air-dried soil on mass basis). Ryegrass (*Lolium multiflorum* L.) was used as a test crop. The Cd concentration in ryegrass in the second and third year was not significantly affected by the rate of organic matter addition in any of the three soils, although the concentration in the first year crop decreased significantly at increasing rate of organic matter addition. The concentration of Cd in ryegrass grown in different soils was in the order: sand> sandy loam> clay loam. The dry matter yield was generally not affected by the rate of organic matter addition but it was higher in clay loam soil followed by sandy loam and sandy soil. Soil pH decreased at increasing rate of organic matter addition. Soil Cd was extracted with 1M NH_4NO_3 and 0.005M DTPA and was also analysed by the sequential extraction procedure. The amount of Cd extracted by both extractants increased with increasing levels of organic matter in all soils. A solid phase transformation of Cd from the oxide fractions to the exchangeable fraction was observed at increasing rate of organic matter addition. The exchangeable fraction of Cd was the highest fraction of the total Cd in these soils.

MYCORRHIZAL INFECTION IN SOME PLANTS FROM PORTUGUESE SERPENTINE AREAS

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The serpentine soils of NE Portugal are known to have high nickel concentrations, high Mg/Ca ratio and nutrients deficiency for plant growth. Seven endemic species of these serpentine areas were collected in the field, in July, and their roots were fixed in alcohol 70% and prepared for mycorrhizal investigation. After clearing (KOH 5%) the roots were stained with trypan blue 0.05% (lactic acid, glycerol, water, 5:1:1) (Philips & Hayman, 1970) and they were observed with an optical microscope. All plant species showed mycorrhizal infection except *Alyssum pintodasilvae*, a Brassicaceae and the only nickel hyperaccumulator species. *Festuca brigantina*, *Anthylis sampaiana*, *Plantago radicata* and *Dianthus marizii* showed AM. In *Reseda virgata* we observed some vesicles but not obvious AM. The morphology and anatomy of the different types of mycorrhiza were clearly investigated. In these species mycorrhizas are important to cope with soil nutrients deficiency rather than to tolerate nickel toxicity.

SOME SECONDARY METABOLITES ALTERED BY HEAVY METALS

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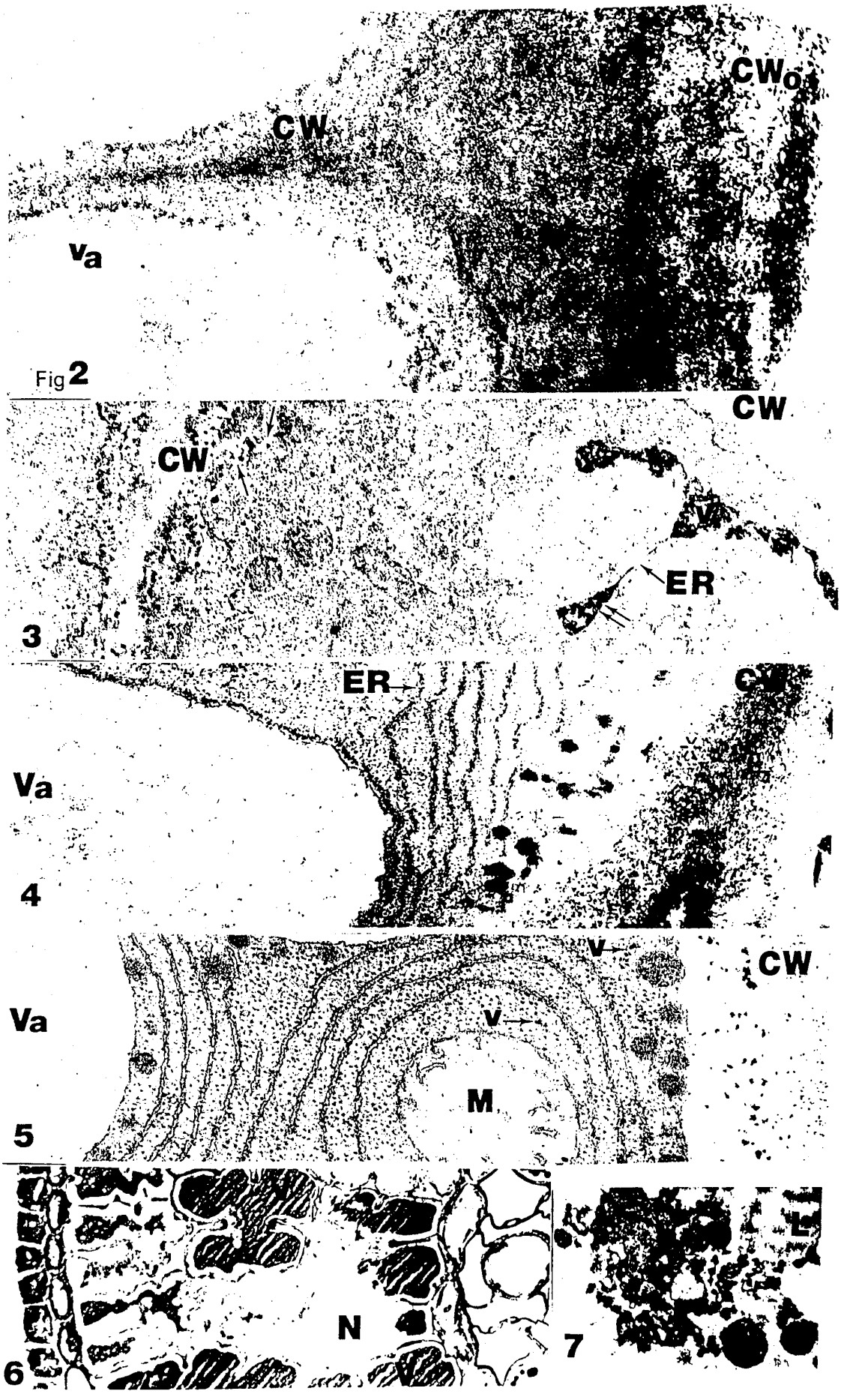
Lead ions were taken up by primary tissues of both root and shoot except for promeristems. Both initials and their derivatives, which give rise to the new shoot and roots, were spared lead. This may explain the lower toxicity of lead to plants than to animals. Initially its uptake was into the cell walls with the highest concentration in the surface part of the cell wall. Next the vesicles and then the endoplasmic reticulum dilated into the vacuoles. After longer treatment the vacuoles contained less lead, suggesting its extrusion from the cell. The concentration of furanocoumarins in *Ruta graveolens* was lowered after two weeks of treatment, and phenolics in pine needles increased in the vacuoles, causing the death of a few mesophyll cells.

INTRODUCTION

Although plants along highways are exposed to different concentrations of pollutants (salt spray, acids, heavy metals) they still manage to survive, sometimes without much damage (ref. 1). What mechanisms of protection and detoxification do plants have which are lacking in animals? We have been looking for reactions of different plant species to various air pollutants. Sprays of acid (ref 2) and salt (ref 3) on pine leaves led to an increase in the concentration of phenolic compounds before cell death, and these are valuable bioindicators of air pollution (ref 4). On the surface of *Ruta graveolens* leaves morphological changes were followed by a decrease in concentration of furanocoumarins (ref 5) known to be protective compounds for the plant (ref 6). While the structure of roots in terrestrial plants makes these organs most suited for absorption and transport of water together with ions dissolved in it (ref 7), uptake of ions can also occur through the leaves (ref 8). Some work has been done on the toxicity of lead ions to the root apex (ref 9), but very little is known about the shoot apex. We intended to compare localization and distribution of lead ions in these apices at the two extremities of the plant.

MATERIALS AND METHODS

Embryonic roots of *Glycine max* (lead-susceptible), and *Allium* (lead-resistant) were compared to shoots of *Ruta graveolens*, where furanocoumarins act as a defense barrier on the surface (ref 10). Germinated seeds of soybean were treated for 48 h with 2000 ppm solutions of $PbCl_2$ of different pH (2.5, 3.5 and 5.5) to evaluate the influence of increased solubility of lead salts on the uptake of lead ions. Radicles were fixed in Karnovsky mixture for electron microscope observation. Root tips were propagated from *A. cepa* bulbs according to the method of Peterson *et al.* (ref 11) and 1 cm-long root tips were treated with different concentrations of $PbCl_2$ or $Pb(NO_3)_2$: 0.2 and 0.3 mg/L, which are sublethal for this plant (ref 12). *R. graveolens* shoots were sprayed for a month with 0.2 mg/L $PbCl_2$, and the furanocoumarin concentrations on the surface of the leaves and in their interior evaluated by HPLC methods (ref 13). One-month-old leaves of *Pinus resinosa* were sprayed as previously described for salt and simulated acid spray (ref 2, 3), and preparations for light and electron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microscopes were made to evaluate structural changes and amounts of phenolic compounds. After lead uptake 0.5 mm shoot apices and, separately, the next 1 mm internode segment, of both rue and pine were squashed. For the root tips autoradiographic methods were used with ^{210}Pb (ref 14).

RESULTS

The root tips and apical internodes of shoots, except for the promeristem, which consists of initials and their closest derivatives (Fig. 1), contained lead, findings consistent with previous observations on root tips (ref 14). In the shoot apices of many plants (ref 15) the initials of the tunica contained phenolic compounds in the vacuoles, and their exterior cell walls were very thick and covered by cuticle, conditions which may be responsible for preventing any ions from entering the cell lying below.

Heavy metals were absorbed by the apoplast before they entered the protoplasts, as evident from deposits in the cell walls (Fig. 2, CW) and the higher concentration of lead in the outermost cell wall facing the surface (CWo). The increased surface of the plasma membrane (Fig. 3, arrow) suggests that endocytosis may be involved in the uptake of lead. A similar suggestion comes from the fact that lead ions are surrounded by the membrane in small vesicles (Fig. 5, v). After a few hours the lumen of the endoplasmic reticulum contained lead (Fig. 3, arrow), which was located in vacuoles arising from the ER (double arrow), but after 24-48 h much less lead was observed in the vacuoles (Fig. 4, 5). The number of lead deposits in the cytoplasm decreased with the pH (Figs. 4, 5). In pine needles dilation of the ER, vacuolization and increased concentration of phenolic compounds in the vacuoles (Fig. 6, v) led to the death of a few mesophyll cells, resulting in small necroses (Fig. 6, n). In such necroses there were conglomerations of cell membranes and phenolic compounds (Fig. 7). The surface structure of the rue leaves was altered. After two weeks a decrease in the concentrations of psoralen, bergapten and xanthotoxin (Table 1), more drastic than after salt and acid sprays, was observed both on the surface and in the interior.

Table 1. *Changes in concentrations* of total furanocoumarins in the whole leaf and on the surface of* Ruta graveolens *leaves.*

Collection date (1989)	Treatment	Leaf position	∑P+X+B† whole leaf	∑P+X+B on surface	% of total on surface
15 March	Nil	Upper	2550±120	410±20	16
		Lower	2000±100	320±15	16
29 March	Nil	Upper	2150±100	620±40	29
		Lower	1600±80	420±20	26
	H_2O	Upper	960±60	450±25	46.5
		Lower	1550±60	980±65	64
	$PbCl_2$	Upper	440±20	170±10	38.5
		Lower	560±40	230±20	41.5

* $\mu g\ g^{-1}$ f. wt; average of two determinations.
† p = psoralen; X = xanthotoxin; B = bergapten.

DISCUSSION

Since plants (including crop species) can tolerate relatively high concentrations of lead (ref 16), sometimes up to three orders of magnitude higher than can animals, they are potentially

hazardous food for humans and other animals. Lead is initially absorbed into roots through the cell walls, following the pathway of water uptake, but not all kinds of tissue incorporate it to the same extent (ref 14). We found that, as the promeristems of both root and shoot are spared these lead deposits, the genetically active initials are protected from chromosomal aberrations which can result even from low concentrations of lead (ref 12). Plants must also have other protective mechanisms, because with increased solubility at a lower pH, lead seemed to be absorbed less by the cytoplasm. The structural difference observed was a thickening of the plasma membrane (ref 17). Similarly, Sieghardt (ref 18) observed no significant damage after lead treatment. These two defense mechanisms should be further investigated to ascertain if they are commonly occurring in all plants, or in resistant plants only. More resistant varieties of one species contained a higher percentage of lead in cell walls than in vacuoles (ref 19). We suggest that this protection of initials is characteristic of plants, which can regenerate from the promeristem, in contrast to animals. This may be the "mystery mechanism" explaining the great differences in resistance to lead poisoning between plants and animals.

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LE DOSAGE DES ELEMENTS TRACES DANS LES LICHENS EPIPHYTES : UNE APPROCHE DE LA POLLUTION ATMOSPHERIQUE

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La pollution atmosphérique, notamment en ce qui concerne les poussières ou matières particulaires en suspension dans l'air devient un problème préoccupant du point de vue de la santé publique. De par leurs caractéristiques biologiques, les lichens en place sur les sites d'observation sont susceptibles de refléter cette pollution tant qualitativement que quantitativement.

Nous avons choisi pour cette étude, deux villes d'une même région, se différenciant pas l'importance de leur urbanisation et leur niveau d'industrialisation. Le but recherché était de mettre éventuellement en évidence des différences quant à la nature et à la concentration relative des éléments traces retrouvés.

Les prélèvements ont été effectués dans des zones concentriques de plus en plus distantes du centre ville ainsi qu'auprès de zones industrielles nettement identifiées. Les dosages ont été réalisés en utilisant l'analyse par activation neutronique instrumentale. Cette méthode permet de doser simultanément une trentaine d'éléments sur un même échantillon, de façon non destructive, dans un domaine de concentration allant de quelques g/kg au µg/kg. Les résultats ainsi obtenus ont ensuite fait l'objet d'une analyse statistique des données.

Nos résultats montrent que les concentrations en éléments traces retrouvées dans les lichens sont de plus en plus faibles au fur et à mesure qu'on s'éloigne du centre des villes ou des zones industrialisées.

L'espèce du lichen recueilli semble influencer la quantité de matière retenue mais n'a que peu d'incidence sur la composition élémentaire des oligoéléments. On constate que cette composition varie suivant les sites de prélèvement et les sources d'émission d'éléments polluants.

Nous avons pu montrer par ailleurs qu'il existait des corrélations fortes entre certains de ces éléments évoquant ainsi la présence de phases minérales déterminées et ce quel que soit l'environnement du lichen.

Cette étude montre bien l'aptitude des lichens à refléter le taux de pollution de l'atmosphère par des particules en suspension et le cas échéant à les différencer entre elles. De par leur durée de vie et leur temps de résidence sur le site, les lichens sont éventuellement capables d'être de bons « intégrateurs » de la pollution.

ARSENIC IN AUSTRIAN SOILS
GEOGENIC CONTAMINATION AND MOBILITY

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Throughout the Austrian Alps mineral deposits of hydrothermal origin containing arsenic minerals or consisting of pure arsenopyrite are present. Springs originating from layers with embedded veins may contain several hundreds of microgram As per liter. For the local population these springs pose no danger, because they are well known as "poison springs".

Aside from these localities with exceptionally high concentrations of arsenic, large parts of the Austrian provinces used as farmland have soils with relatively high concentrations of As. During the ice ages glaciers eroded much of the Alps including arsenic-rich ore deposits. The erosion products were transported over large distances and were deposited in valleys and regions, that now are used extensively for farming and animal production. For example, in the provinces of Salzburg 12% of the soil samples investigated exceed the threshold value of 50 mg/kg As. These arsenic concentrations were determined in extracts obtained by treating the soil with aqua regia. Because most arsenic compounds including insoluble, not bioavailable compounds are dissolved by this strong oxidizing agent, concentrations of arsenic found by this method cannot be used to predict arsenic concentrations in plants, evaluate the potential contamination of ground water, and generally estimate risks to exposed populations.

To study the transfer of arsenic from the soil to food, a program was established in 1994. In cooperation with an experimental farm, the uptake of arsenic by agricultural plants under several natural conditions from different soils is explored. In soils and plants total arsenic is determined and arsenic compounds are identified and quantified by HPLC-ICP-MS. Preliminary results show, that grass growing on slightly contaminated soils converts inorganic arsenic to organic arsenic derivatives. The less toxic organic arsenic compounds must be considered in assessments of risk. In addition to the field studies, several experiments are carried out in the laboratory mainly to study the physico-chemical behaviour of arsenite and arsenate in soil. First, a suitable extraction procedure for arsenic must be found, that produces concentrations of extractable arsenic in the soils correlating with the concentrations found in plants. In the literature more than 40 extraction mixtures are reported for inorganic arsenic species alone. Experiments with montmorillonite as a modell for clay minerals show that the extractable proportion of arsenic could vary between 10 and 90% of the total adsorbed As depending on the nature of the extraction mixture. Surprisingly, arsenate is much more extractable than arsenite. This different behaviour of the two oxidation states is supported by infrared-spectroscopic studies. The characteristic vibrations for a Ca-montmorillonite-arsenate are located at lower frequencies than the vibrations for the arsenite system. These studies indicate a stronger bonding of arsenite compared to arsenate for this model system.

INFLUENCE OF MINING ACTIVITY IN THE SOME HEAVY METAL CONTENTS IN SOILS AND PLANTS

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Four mining zones were chosen for this study in the province of Salamanca (tin and tungsten ores with sulphides hosted in sales and granites).

In soils of Salamanca province arsenic background is less than 10 ppm, anomalous soils contain more than 10 ppm and range from 10 to > 1000 ppm, due mainly to arsenopyrite alteration in mining areas.

High and very high arsenic contents in plants (even 40 ppm some of them probably phytotoxic) were obained at these mining zones. Arsenic contents in *Vulpia bromoidea* (L) S.F. Gray, shows a correlation with arsenic contents in their soils; nevertheless for *Agrostis castellana* Bois & Rent other pedological factor must act (pH, probably) more than arsenic content in soil. *Dactylis glomerata* L. presents a higher degree of arsenic accumulation than *Ag. castellana* and *Cynosurus echinatus* L. in the same As-anomalous soil.

On the other hand, selenium contents in plants are low, with most part of data showing values lower than 50 ppb (considered a deficitary content for animal nutrition) and ranging from 2 to 260. These values are in agreement with other previous data in the Salamanca province. *V. bromoides* shows higher selenium contents than *Ag. castellana* in most of the soils studied.

EFFECTS OF SOIL CONTAMINATION ON FOREST STATUS NEAR A NICKEL SMELTER

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The concentrations of nickel and copper in soils and plants in the vicinity of the nickel smelter at Nickel, Russia, are approximately one to two order of magnitude higher compared to background levels in the region. The organic horizon of podzols is the important accumulator of heavy metals and barrier against transport of pollution to underlying mineral horizons. When this horizon become overloaded adverse impacts on vegetation occurred.

Background and objectives. The areas surrounding the nickel smelter in Nickel, Russia, are heavily polluted from emissions of heavy metals and sulphur dioxide for several decades. According to data from Laboratory on Observation for Environmental Pollution in Nickel, the emissions from the smelter are 136 tons of nickel, 92 tons of copper and 4.8 tons of cobalt. The total annual deposition of nickel and copper reaches 100 mg/m^2 near smelter, decreasing significantly within the first 10 km. In background areas it has been estimated to about 1-3 mg/m^2 (Sivertsen et al. 1992). The objectives of this study were to estimate the heavy metal contamination of soils in the vicinity of pollution sources, to assess possible effects on forest vegetation and to reveal the significance of soil organic horizons in protection of plants and mineral horizons against pollution.

Approach. The study area is located to areas around a nickel smelter in the north-western part of the Kola Peninsula, Russia, close to the Norwegian border. Plant and soil samples were collected from pine and birch forests at different distances and directions from the smelter. Total amounts of heavy metals in soil and plant samples were determined by use of X-ray fluorescence (Russian samples), and by ICP-AES (Norwegian samples). Acid-soluble and ammonium nitrate-extractable heavy metals in soils were determined by AAS (Russian samples) and ICP-AES (Norwegian samples).

Main results. Soils close to the Nickel smelter contained higher concentrations of both nickel and copper compared to remote sites. Close to the smelter the concentrations of heavy metals in the organic horizon were in the range 32-40 mmol/kg (total), 9-33 mmol/kg (acid-soluble), and 8-25 mmol/kg (ammonium nitrate-extractable). At the most exposed sites also iron, manganese, chromium, strontium and arsenic were found to be at elevated concentrations. At the most remote site (40 km from the smelter), the concentrations of heavy metals were approximately 1.3-2.3 mmol/kg. The heavy metals were significantly concentrated in organic horizons. The mobility of Ni and Cu in the soils was limited, as shown by a sharp reduction in their concentrations with depth. However, the concentrations of Ni and Cu in mineral horizons were higher in the vicinity of the smelter than in sites remote of the smelter (Koptsik & Niedbaiev 1992). The total amount of heavy metals alone is not enough for assessment of the environmental impact of polluted soils. The determination of metal species in solution is important to evaluate their behavior in the environment and their mobilization capacity. The acid-soluble metals accounted for 1-45% of the total content in the organic horizons in background sites and increased significantly in polluted sites near the smelter (50-97%). The percentage of total metal content associated with exchangeable fractions in organic horizons were 0-29% and 40-77% in background and polluted plots, respectively. Heavy metal content reached 18-25% of the soil CEC in heavily polluted soils.

Concentration of pollutants in pine needles and bark were increasing largely with decreasing distance from the smelter. Previous year needles were richer in heavy metals than current year needles.

Impacts of heavy metals on vegetation depend on total input, metal availability, mycorrhizal tolerance and plant tolerance for the metals. Acid sandy podzols with low content of organic matter have the lowest ability to buffer metal inputs by transforming them into unavailable forms. Acid deposition can change the availability of heavy metals. The levels of heavy metal input which can result in toxic impact on plants are highly variable. Critical toxic level ranges at 50-100 and 60-125 mg/kg for Ni and Cu, respectively, have been documented for agricultural plants (Alloway 1990). Cu becomes phytotoxic when its level exceeds 5% of the soil's CEC (Mathur and Levesque 1983). According to this, toxic effects to vegetation caused by deposition of air pollution from the nickel smelter may occur within 25-35 km from the smelter, depending on prevailing wind directions. The total content of Ni and Cu in the soil organic horizons exceed 130 and 80 mg/kg, respectively, and their fraction at the soil's CEC reach 2%. However, vegetation damage in these areas may mostly be caused by direct effects of sulphur dioxide. Visible symptoms caused by sulphur dioxide in this region are reported by several authors (see inter alia Aamlid 1993 for colour photographs). Other implications of the heavy metal deposition and soil storage of heavy metals might be important for animals using different plants as forage (Sivertsen et al. 1992). A certain uptake in berries is also reported in the studied area (Barkan et al. 1990; Kruglikova 1991; Aamlid & Skogheim 1993). Elevated heavy metal concentrations may be caused by both root uptake and by direct deposition to plant parts. Different plant species may have their roots distributed in different soil depths, and therefore respond differently to contaminated soils (Aamlid & Skogheim 1993; Løbersli 1991).

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CELLULASE ACTIVITY IN HEAVY-METAL-POLLUTED SOIL

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The heavy metal contaminations in agricultural arable land keep increasing due to the return of sewage sludge into agriculture land, application of special fertilizers, utilization of agricultural chemicals such as Bordeaux mixture, etc. Recently, this is aggravated by the heavy metals contained in the household waste water and the atmosphere and the various heavy metals emitted from the high technology industries. The heavy metals once contaminated in soils are not easily leached but accumulated in the soils. Since the heavy metals toxic to organisms, Scientists realized that they might affect the soil microbe ecosystem. However, there are very few researches that clarified these problems about them.

Cellulase is an important indicator of the carbon metabolisms in the soil, since it is as an enzyme to decompose the cellulose which is the most aboundantly existing organic compound on the earth. So, this study was conducted to clarify the effects of heavy metals on cellulase activity.

These experiments investigated total and extractable heavy metals (Cd, Zn, Cu) in contaminated areas (Toyama, Ohta, Tsushima) in Japan and their effects on the activities of exocellulase and β-glucosidase in the cellulase multi-enzyme system.

The results obtained are as follows:

1) The ratios (heavy-metal-content of each area/natural content) were on average: Toyama soils Cd 3.0, Zn 4.2 and Cu 1.2; Ohta soils Cd 6.5, Zn 6.6 and Cd 28.0; Tsushima soils Cd 23.0, Zn 20.0 and Cu 3.0. The degree of heavy-metal-pollution was in the following order: Tsushima > Ohta > Toyama soils.

2) Exocellulase and β-glucosidase activities were decreased with increasing the heavy metal content in all of 3 areas. Especially, very high negative coralation between the heavy-metal-content and the cellulases (exocellulase and β-glucosidase) activity was observed only in Ohta soils, since the soils were sampled from a paddy field with small spacial variability.

3) It was found that β-glucosidase activity was more sensitive to heavy metal polluted soil than exocellulase activity.

4) The extraction way of heavy metals (0.1 N HCl extractable and total) made no difference on the relation to the exocellulase and β-glucosidase activities.

5) Even if high concentrations (10,000 ppm) of Cd, Zn and Cu were added to soil, the exocellulase and β-glucosidase activities do not decline. This fact states that Cd, Zn and Cu do not inhibit cellulase activity. Thus, it can be estimated that heavy metals are incorporated into microbes, disorder thier metabolism, thereby affect the biomass quantitatively, in turn affects soil cellulase concentration.

FORMS OF HEAVY METALS IN FOREST SOIL PEDONS, ONTARIO, CANADA

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Forest decline has affected large areas of upland coniferous and lower altitude hardwood forest in Central Europe and Eastern North America over the past 10-15 years. In Canada the worst damage has occurred in the sager maple hardwood forests of Southern Quebec and Central Ontario and in montane red spruce. Although the exact causes of forest decline are yet unknown, the effects of acid deposition, low soil pH, and aluminum toxicity to tree roots are suspect factors. It is known that the mobility and phytoavailability of heavy metals in soil increase with decreasing soil pH. This increase in turn may be related to changes in the distribution of metal forms in soil constituents.

This research was conducted in Central Ontario - sager maple forest, where the decline has been occurring but not yet severe. The distribution pattern of the forms (exchangeable, carbonate, Fe-Mn oxides, organic, and residual) of Cd, Cu, Ni, Pb, and Zn in the forest soil pedons from Loring and Frost, Ontario, Canada was studied. These data showed that soil acidification led to some changes in soil chemistry, including marked alterations in the occurrence of metal forms, which in turn may affect nutrient and metal bioavailability and subsequently influencing forest decline.

THE INFLUENCE OF CHLORIDE ON THE CADMIUM UPTAKE BY SWISS CHARD (*BETA VULGARIS* L. CV. FORDHOOK GIANT). II. A RESIN-BUFFERED SOLUTION CULTURE EXPERIMENT

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It has been demonstrated under field conditions that increasing concentrations of salt (NaCl) in soil increase the phytoavailability of Cd (McLaughlin *et al.* 1994). This effect has been demonstrated to be due to chloro-complexation of Cd increasing total Cd concentrations in solution (Smolders *et al.* 1995). It is normally assumed that it is the activity of metal ion in solution which controls plant uptake of trace metals (Chaney 1988). This experiment was designed to test the hypotheses that chloro-complexation of Cd can enhance plant Cd uptake by:

(a) increasing the diffusion of free Cd^{2+} ion through soil to the root surface (Chaney 1988), or

(b) increasing concentrations of $CdCl^{n}_{2-n}$ species in solution which are also absorbed by roots in addition to Cd^{2+} species.

Swiss chard (*Beta vulgaris* L., cv. Fordhook Giant) was grown in complete nutrient solution continuously recirculated over a chelating resin (Chelex 100). The resin was loaded initially with Cu, Zn, Mn and Cd (spiked with ^{109}Cd). Treatments were increasing concentrations of Cl (0.01, 40, 80 and 120 mM) in the nutrient solution with four-fold replication. To isolate effects of ionic strength on Cd^{2+} activities (and osmotic stress for plant roots), solution Na concentrations and ionic strengths were equal in all treatments by compensating with $NaNO_3$. After germination, plants were grown for 10 days on half-strength Hoaglands solution prior to transplantation to the resin-buffered solution. Each container contained two plants growing in one litre of nutrient solution with trace metal activities buffered by passing nutrient solution (10 ml min^{-1}) over a single column of resin. Plants were harvested after 10 days growth in the resin-buffered solutions. After harvest, plant material was separated into roots and shoots. Toots were washed for 30 min in a solution containing unlabelled Cd. Plant materials were dried (70°C) prior to determination of ^{109}Cd activity. No nutrient deficiency symptoms were visible on the plants. Dry weight of both roots and shoots was unaffected by increasing Cl concentrations in solution (data not shown). Activities of Cd^{2+} was not significantly ($P>0.05$) affected by increasing concentrations of Cl in solution. In contrast, total Cd concentrations in solution increased significantly ($P<0.001$), from 12 nm at 0.01 mM Cl to 47 mg kg^{-1} to 106 mg g^{-1} (Figure 1). The results indicate that the effect of Cl in enhancing Cd uptake in soils need not be related only to enhancement of diffusion of Cd^{2+} through soil to the root, but suggest either:

(a) Cd-Cl species in solution are also phytoavailable (in addition to Cd^{2+}), or

(b) Cl enhances diffusion of Cd^{2+} through the unstirred liquid layer adjacent to the root surface or through the apoplast to sites of Cd uptake within the root itself.

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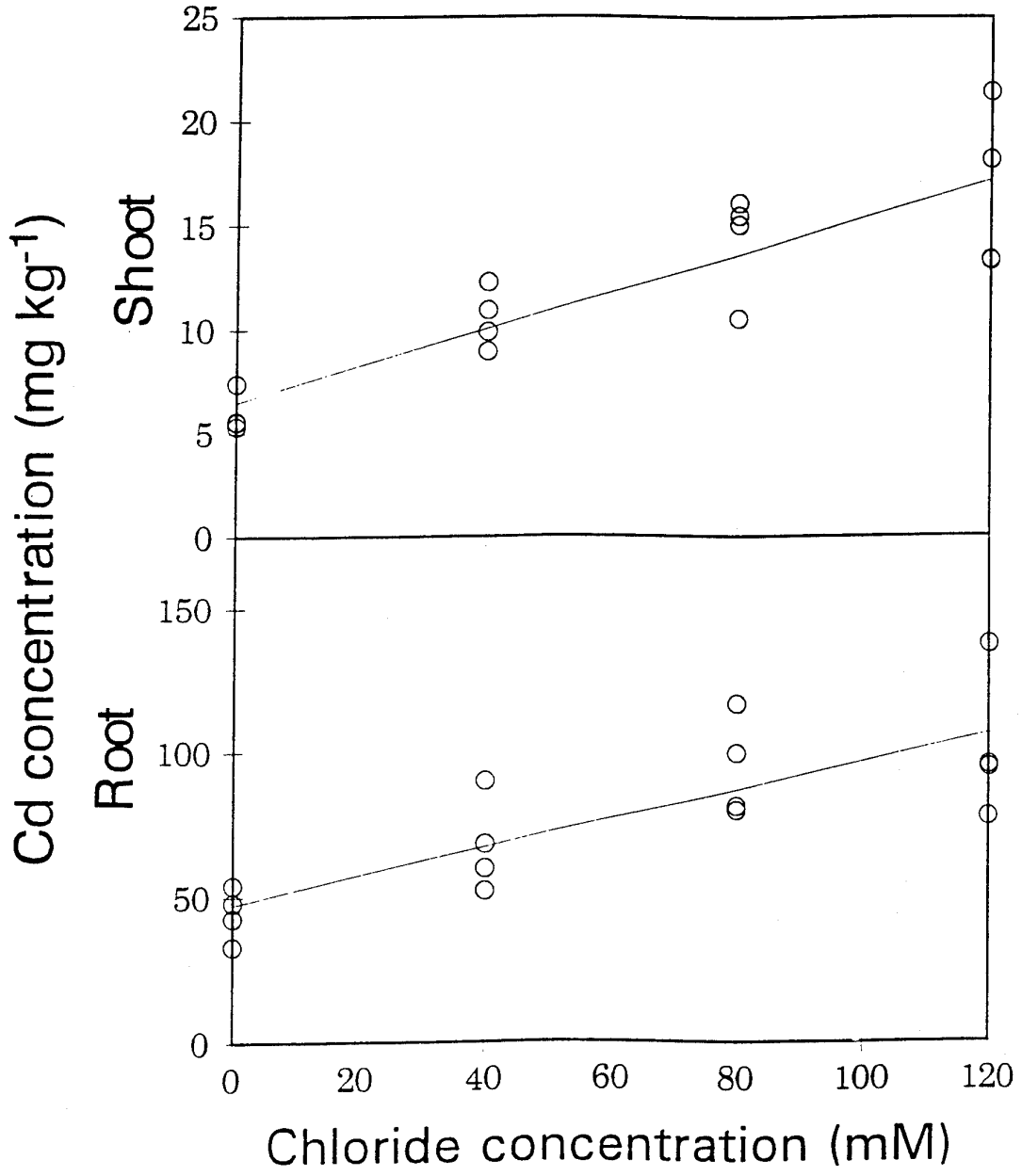
Figure 1. Concentrations of Cd in roots and shoots as affected by Cl concentrations in nutrient solution. Circles are actual observed data and the solid line is the fitted linear regression line. Salt concentrations normalised to 120 mM in all treatments using $NaNO_3$.



THE DYNAMICS OF SOME TRACE ELEMENTS IN A FOREST ECOSYSTEM

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The aim of the present work is to gain insight into the state of some trace elements in the biogeochemical cycle of a forest ecosystem of *Quercus pyranaica* situated in the South of the province of Salamanca (Sierra de Gata, Central-Western Spain) with a view to evaluating the presence of such elements and predicting, perhaps, possible deficiencies.

The study was carried out in two climosequences of soils of similar characteristics, humic Cambisols with rainfall as a variation factor, analyzing the effects of the latter on two different parent materials: a two-mica granite and slate.

The range of variation in rainfall between both sites is similar (granite: 720-1500 mm; slate: 774-1200 mm), for which available Fe, Mn, Zn and Cu in the soil were determinated by extraction with DTPA, pH=7.3, by acid digestion of total elements.

The values obtained for these available elements in the two climosequences reveal that in general the element contents decrease with depth, as would be expected for this type of soil, observing much higher contents in the surface horizons owing to the greater amount of surface organic matter and the affinity of these trace elements for this. Similarly, a decrease in these elements parallel to the increase in rainfall is seen, this decrease being much more marked in the upper horizon and for Mn, among the elements considered. At the same time, this trend is better observed in the climosequence on granite.

With respect to total elements, it is seen that the effect of rainfall on them is not so clear. Additionally, as for the available elements, a clear relationship with the lithology can be appreciated, the contents of these elements being higher in the soils on slate.

ACCUMULATION OF HEAVY METALS IN PLANT TISSUES AND THEIR USE AS BIOINDICATORS IN CAIRO, EGYPT

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Experimental plants of barley (*Hordeum vulgare*) and lettuce (*lactuca sativa*) were transplanted and exposed to the atmosphere of Cairo various districts. Results revealed a negative significant relation between air pollution levels and all examined growth parameters. Total chlorophyll contents in both plants were decreased with the increase of pollutant concentrations.

A straight line relationship has been established between total sulphation dose and sulphur concentrations in plant tissues. Moreover, it was found that each crop plant has its own characteristics in the accumulation of atmospheric metals. The accumulation of the investigated metals (Pb, Cd, Zn, Cu) in plant tissues were significantly dependent on their concentrations in the atmosphere and the duration of exposure to these pollutants. Consequently, it is concluded that barley and lettuce plants can be taken as bioindicators for sulphur and heavy metals pollution in polluted urban areas.

The following relationships were established between metals exposure dose (the concentration of the metal in air x duration of exposure in days) and their concentrations in plant tissues (dry basis):

Pb concentration (in barley) = 0.62 x Pb exposure dose + 24.7.
Pb concentration (in lettuce) = 0.43 x Pb exposure dose + 69.9.
Cd concentration (in barley) = 1.4 x Cd exposure dose + 1.3.
Cd concentration (in lettuce) = 1.04 x Cd exposure dose + 2.3.
Mn concentration (in barley) = 1.3 x Mn exposure dose -2.1.
Mn concentration (in lettuce) = 1.3 x Mn exposure dose - 8.1.
Cu concentration (in barly) = 2.2 x Cu exposure dose + 25.3.
Cu concentration (in lettuce) = 1.7 x Cu exposure dose + 28.9.

These results show that barley and lettuce can be used as bioindicators for pollution with several heavy metals.

CONTAMINATION OF THE AGRICULTURAL LAND DUE TO INDUSTRIAL ACTIVITIES SOUTHERN OF GREATER CAIRO

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The industrial processes in the investigated area include ferrous and non-ferrous metallurgical work, ceramics, fire bricks, cement industry, etc. Soil, weeds, vegetation and dust samples were collected and analysed for several heavy metals. High levels of Pb, Cd, Ni, Cr, Mn and zinc were found in the soil close to a lead, zinc smelter. In the heavy contaminated area concentrations of more than 500 μg/g Pb, 1200 μg/g zinc and 50 μg/g Ni and Cd were recorded in the surface soil at 1500 m from the smelter. The concentrations of the heavy metals in the contaminated area (3-5) km from the smelter) reached more than 200 μg/g Pb and 25 μg/g Ni and Cd and 160 μg/g zinc. Higher levels of these toxic elements were found in the dust on the leaves of the examined vegetations. Furthermore, accumulated concentrations reached more than 100 μg/g Pb and 10 μg/g Cd in leaves of herbs and maize. Soil close to cement industry found enriched with heavy metals but it is much less pronounced.

EFFECTS OF URBAN AIR POLLUTION ON PLANT GROWTH AND THEIR USE AS BIOINDICATORS IN CAIRO, EGYPT

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Experimental plants of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa*) were transplanted and exposed to the atmosphere of Cairo various districts. Results revealed a negative significant relation between air pollution levels and all examined growth parameters. Total chlorophyll contents in both plants were decreased with the increase of pollutant concentrations in the atmosphere.

A straight line relationship has been established between total sulphation dose and sulphur concentrations in plant tissues. Moreover, it was found that each crop plant has its own characteristics in the accumulation of atmospheric metals. The accumulation of the investigated metals (Pb, Cd, Zn, Cu) in plant tissues were significantly dependent on their concentrations in the atmosphere and the duration of exposure to these pollutants. Consequently, it is concluded that barley and lettuce plants can be taken as bioindicators for sulphur and heavy metals pollution in polluted urban areas. The effect of plants exposure to air pollution in Cairo urban area on sugar and nitrogen contents as well as amylase activity, GOT and GPT were also studied. Several conclusions and recommendations are included.

HEAVY METAL CONCENTRATIONS IN NATURAL VEGETATION IN RELATION TO ATMOSPHERIC HEAVY METAL DEPOSITION

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Long-range atmospheric transport of heavy metals from other part of Europe has led to an extensive contamination of the organic surface layer of natural soils in areas of Southern Norway. Enhanced levels by more than a factor of 10 (Pb), 5 (Cd), and 3 (Zn) are observed relative to areas of Norway not appreciably exposed to air pollutants (Allen and Steinnes, 1980; Steinnes *et al.*, 1989). Corresponding differences have been reported in some higher plants (Solberg and Steinnes, 1983).

In the present work Zn, Cu, Pb and Cd concentrations were examined in several plant species and corresponding organic topsoil collected in forest and on ombrotrophic bogs along a temporal (1982-1992) and a spatial (Southern Norway - Central Norway) heavy metal deposition gradient. This allowed relations between metal concentrations in plants, soil/peat and spatial and temporal changes in atmospheric deposition of heavy metals to be studied. Pb concentrations in plants decreased significantly from 1982 to 1992 both in Southern and Central Norway, while stable levels were observed in surface soil. These observations together with a decrease in atmospheric Pb deposition rates of about 70% both in Southern and Central Norway, proves that direct atmospheric deposition strongly influenced Pb levels in vegetation. Pb concentrations in vegetation were significantly higher in Southern Norway than in Central Norway both in 1982 and 1992, evidently due to a more than 15 times higher Pb deposition in Southern Norway. Plant concentrations of Zn, Cu and Cd were significantly higher in Southern Norway than in Central Norway both in 1982 and 1992, most likely because of higher root uptake in Southern Norway due to the distinctly higher surface soil levels of Zn, Cu and Cd from air pollution in this region. No distinct changes in Zn, Cu or Cd concentrations in plants, however, were observed from 1982 to 1992, even in Southern Norway where wet deposition of these elements decreased by about 26% (Zn), 40% (Cu) and 51% (Cd) during this period of time.

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JOINT EFFECTS OF CHROMIUM AND PHENOL ON THE QUALITY OF RICE AND RELEVANT FOOD-SECURITY INDEXES

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Rice is often exposed to the combined pollution of chromium and phenol in China and other developing countries. In order to assess human health risk of the toxic chemicals in food and to improve the quality of export food from developing countries, interrelationships between joint effects of chromium and phenol from rural enterprises and the low quality or rice were studied using the pot-culture experiment combined with the computer simulation, on the basis of the on-the-spot investigation on the suburbs of Shaoxing city, Ningbo city and other cities in the south of China. The research indicated that there were some differences in the accumulation and critical contents of the toxic chemicals and the concentration of essential composition in rice affected by the interaction between Cr and phenol, compared to single-factor effects of chromium or phenol. The reliable food-security indexes related to joint effects of Cr and phenol in agroecosystems were suggested according to these differences.

CONTRIBUTION OF DIFFERENT CHEMICAL FORMS OF NATIVE AND APPLIED ZINC TO ITS BIOAVAILABILITY ON HIGHLY CALCAREOUS SOILS*

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Zinc, being an essential element for plant growth, becomes toxic if excess of it is absorbed. Different forms of zinc are found in soil but not all of them have the same bioavailability. This study was conducted to compare the bioavailabilities of different chemical forms of native and applied zinc on highly calcareous soils. The experiment was a 20 x 3 factorial in triplicate, with 20 highly calcareous soils of Iran (pH 7.9 to 8.5; calcium carbonate equivalent 16 to 58%) and 3 levels of previously-applied zinc (0, 10 and 20 mg Zn/kg as zinc sulfate). Maize (*Zea mays* L.) was grown for 7 weeks in pots containing 1500 g soil under greenhouse conditions. Soil samples, taken from pots before planting, were used for determination of exchangeable, sorbed, organic, carbonate, and residual (sulfide) forms of zinc by sequential extraction method. Zinc uptake (dry matter x zinc concentration) of plant tops was taken as the index of bioavailability, i.e., the amount of zinc available under the conditions of the experiment. Multiple regression analysis showed that while percent contribution of carbonate form to plant zinc uptake was 60.1, similar figures for exchangeable, sorbed, organic, and residual forms were 7.1, 0.4, 0.4, and 0.0, respectively. Thus, it may be concluded that, in bioavailability of both native and applied zinc of highly calcareous sois, carbonate zinc is the most influential form followed by exchangeable form and that the influence of other forms are nonsignificant.

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CONTAMINATION OF HERBACEOUS COVER, OAK LEAVES AND PINE NEEDLES WITH SOME METALS (Fe, Cr, Cd, Pb and Zn) AROUND AN INDUSTRIALISED AREA OF IZMIR, TURKEY

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Presently, the effects of heavy metal pollution in the environment is gaining importance in Turkey too due to heavy industrialisation. Besides traffic, local source emissions of iron-steel industries are creating heavy pollution problems in urban and suburban environment. One of them is located in the northwest of the third biggest city, Izmir in Turkey with about 3.5 million inhabitants and represents a rather industrialised area having five large iron-steel mills but also areas of forestry and agriculture. The aim of the present study is i) to determine contamination of herbaceous cover, oak leaves and pine needles with Iron (Fe), Chromium (Cr), Cadmium (Cd), Lead (Pb) and Zinc (Zn) near largest iron-steel mill and ii) to determine variation of pollution with distances from the mill. For this purpose, above ground part of herbaceous cover, oak leaves and pine needles were taken on five occasions between the dates 26.02.1992 and 13.08.1992 at distances of 0.25 km, 1.0 km and 2.5 km along a transect in a easterly direction from the mill which is the largest one in the area with a 5000 ton/day steel production capacity.

The measurements were made by aspirating the sample solutions to a Pye-Unicam sp 9 atomic absorption spectrophotometer. The quality of the chemical analyses was checked by analysing blanks, duplicate samples and recovery studies.

The results show a contamination of all six metals with a difference among the plants investigated and a decrease with the distance from the mill. At distances of 0.25 km, 1.0 km and 2.5 km, the Fe in the unwashed leaves samples of the plants ranged from 100 $\mu g g^{-1}$ dw up to 4938 $\mu g g^{-1}$ dw, Cr from 3.0 $\mu g g^{-1}$ dw to 1.0 $\mu g g^{-1}$, Cd from below detection limit to 16.3 $\mu g g^{-1}$ dw, Pb from 20 $\mu g g^{-1}$ dw to 500 $\mu g g^{-1}$ dw and Zn from 21 $\mu g g^{-1}$ dw to 938 $\mu g g^{-1}$ dw.

The relative standard deviations (n=5) and recovery of the measurements of the elements analysed were found to vary between 0.9-6.8% and 91-98% respectively. The arithmetic means of the measured heavy concentrations in herbaceous cover were generally higher than those in oak leaves and pine needles indicating that herbaceous cover is more effective for accumulation of heavy metals investigated.

In conclusion all data obtained in this study show that the study area within 2.5 km of the iron-steel mill is heavily polluted by heavy metals. Stack emissions of the mill which mainly consist of particulates loaded with metals have caused regional "forest" decline. Also, agricultural activities in the area have been adversely affected. If steps cannot be taken to prevent further pollution by the mill, beside environmental deterioration, threat to human health will increase considerably.

EFFECT OF PHOSPHORUS ON THE DISTRIBUTION IN SOIL AND PLANT UPTAKE OF SOME HEAVY METALS (Zn, Fe, Mn, and Cu)

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A study was undertaken on the influence of phosphorus fertilization on the distribution among soil fractions and plant availability of Zn, Fe, Mn, and Cu.

In a pot experiment increasing doses of $Ca(H_2PO_4)_2$ were added (0, 100, 200, 350, 500 mg P/kg) to a non polluted and Zn enriched (100, 250 mg Zn/kg) heavy loamy sand. Following a greenhouse experiment, the soils were sequential fractionated into water soluble, non specifically adsorbed-exchangeable, specifically adsorbed-exchangeable, organically bound, Mn- and Fe- oxide bound and residual fractions of heavy metals.

Relation among heavy metal fractions and their plant uptake was evaluated by correlation and regression analyses.

In the conditions of a pot experiment with oats followed by mustard as the after-crop carried out, the effect of the applied phosphorus fertilization on the plant availability of heavy metals was not uniform and depended on the metal and, in same cases, also on the plant.

ON THE BIOCHEMICAL PROCESSES IN MOUNTAINS OF SOUTH-WESTERN BULGARIA

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It is well known that the aggravation of the forest ecosystems is a consequence of complex factors which relatively have changed depending on the specific conditions and is connected partly with the increasing of heavy metals content in the air, water and soil because of the anthropogenic activity.

In the article the results of the complete investigations on the macro- and microelement content of soil and plants of Rila, Pirin and Vitosha mountains situated in the south-west part of Bulgaria are presented and have the aim to determine the chemical elements accumulation in plants.

Geographically the area is specific because of the location of the highest peak on the Balkan peninsula- peak Mussala /2925 m/, which could make possible the evaluation of transborder atmospheric transfer of global, regional and local origin.

The purpose of the provided investigations is to estimate the heavy metals pollution in that area and the connected with it destructive processes in the forest ecosystems.

The distribution and the dynamic of 15 chemical elements in plant species from the near ground layers is discussed. Special attention has been given to the spruce ecosystems.

Retrospective monitoring has been made on the basis of dendrochronological chemical analyses of tree rings of representative subendemic plants and showed a tendency toward increasing of Zn, Fe, Al, Mn, S etc. concentrations during the last years and for Pb, Sr, Ba, Ni etc. for the period 1952-1971.

The analyses have been carried out by ICP - AES techniques.

BIOGEOCHEMICAL FOOD CHAINS OF THE SOUTH FERGANA SUBREGION OF THE BIOSPHERE AND THEIR CORRECTION

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The biogeochemical provinces as atypical ecosystems are differing from neighboring territories by the level content of chemical elements or associations in them and, thus causing different biological reactions of some part of regional flora and fauna [2, 9]. The biogeochemical provinces may be viewed as natural, natural-technogenic and technogenic. Taking into account hazard ecological situation of some polymetallic ones the modern studing of azonal and zonal biogeochemical provinces and endemies is prime importance [4, 5, 6]. Therefore complex biogeochemical studies of the four mercury-antimony and mercury ore manifestations in the northern slopes of Turkestan and Altai mountain ridges started within the Central Asian region of biosphere in the 1980 have been carried on using the comprehensive ecology methodology and special methods of AAS and gas-liquid chromotography [3, 8].

The deposits are low temperature hydrothermal ones within the contact of limestones and shales. The rocks S_1-D_2 serve as screening rocks. Such minerals as cinnabar, realgar, fluorite, antimonite, orpiment are widespread. The major part of calcareous rocks is represented by dark-brown soils on loess-like loam with gravel pebbled deposits underlying it, and dark grey soils. Natural waters of the regional mountain rivers with pH ranging from 6.5 to 8.0 are poorly mineralized.

Maximal local concentrations of mercury, antimony and arsenic in the subregional soil amounts to 360, 6470 and 308 ppm, respectively. Wide range of natural concentrations of elements in the mantle of soil permitted the character of mercury, antimony and arsenic accumulations by plants and microorganisms in soils to be studied. It was stated that from 0.3 to 160 ppm of mercury, from 8.8 to 1560 ppm of antimony and 2-250 ppm of arsenic may be related to the microbic air-dry biomass. The accumulation of arsenic by the microbic biomass as compared to that mercury and antimony is much less pronounced. Coefficient of biological absorption for arsenic is 0.16-2.07, while for mercury and antimony it ranges from 0.26-36.64 and 0.40-73.19, respectively [8]. Higher plants contain 0.1-184 ppm of mercury, 0.3-750 ppm of antimony and 0.1-80 ppm of arsenic of air-dry mass, that is much higher that the existing tolerant levels of mercury in feedstuffs [7]. The relations of Hg-concentration estimated by means of correlative analysis were the following: soil-microflora ($r=+0.849$), biomass of soil microorganisms-mobile Hg-forms in soil ($r=+0.771$), soil-mobile forms ($r=+0.590$), soil-plant ($r=+0.504$), plant-soil microflora ($r=+0.503$), mobile Hg-forms-plant ($r=+0.281$). Thus, the correlative relation between the Hg-concentrations in the soils and the Hg content in the biomass of soil microorganisms is the most considerable.

In the biogeochemical mercury provinces of Central Asia the farm animals have symptoms of chronic mercury toxicosis, characterized by the decrease of hemoglobin, of katalase, peroxidase and glutathionperoxidase activity, by the blocking of thiogroups, wide accumulation of mercury by the organs and tissues and metallothioneine synthesis and by the disturbances of calcium-phosphorus metabolism. Sodium thiosulphate proved to be effective means in mercury detoxication in the organism of animals [3, 8]. The higher content of Hg in liver of frogs were accompanied the activation of metallothioneine synthesis (to 4000 ppm) and increasing of hemopoesis [3, 8]. In case of a high content of mercury in soils, pasture plants, biomass of soil microorganisms, in organs and excrements of farm animals from

biogeochemical mercury provinces methylmercury was not detected. This form of mercury was found to occur mainly in water, sediments, water organisms, amphibious, some insects and reptiles. The part of total mercury as methyl form accounts averagely for 46, 28 and 8 per cent in the fresh fish, frogs and lizards respectively. Up to 20% of mercury migrates as methyl derivative in natural waters especially during the period of maximal biological production in water basins. Alkylmercury content in water basins of mercury provinces is only from 1.5 to 2.0 times higher than methylmercury level in natural waters of the European biosphere subregion.

The obtained data show the domination of mercury transformation into methylmercury in water medium. Desalkylation of methylmercury exists in the land resulting in the detoxication of mercury as sulphide. The mercury methylation seems to be like the process that dominated under the conditions of primary atmosphere and hydrosphere of the Earth which had reduction character. Further evolution of organisms resulted in the loss of this mechanism, particularly during the period of mercury high liberation from the interior part of the Earth. In this case the major part of organisms was capable for desalkylation of methylmercury synthesized in the biosphere either biogenetically or abiogenically. It is this biogeochemical process that we found in land organisms [3, 8].

The man pathology of mercury biogeochemical provinces is studied slightly. There are the information that higher mercury and antimony level in diet of population of this territory effects on developement of endemic goitre immunological seakness and hard form of anemia [1, 8]. With aim reabilition of ecological situation the application of special measures is very needed (for example, the allotment of sodium thiosulfate, methionine or other sulfur compounds and immunomodulates. Nevertheless according to biogeochemical criteria [5] the biogeochemical provinces of the South Fergana subregion of the biosphere is attributed to a zone of ecological crisis or disaster ones.

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DYNAMICS OF Fe, Mn, Cr AND Pb IN CONDITIONS ENVIRONMENT POLLUTION BY HEAVY METALS

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In the present investigation the dynamics of heavy metals / Fe, Mn, Cr, Pb / accumulation and distribution in organs of maples / *Acer platanoides* L., *Acer pseudoplatanus* L., *Acer negundo* L., *Acer saccharinum* /, growing on the polluted and non polluted areas, was analysed. The metal level in each organ /root, branch, bud, leaf/ of the plants from the zone of industrial emissions is higher than in the control one. Especially it concerns the strongly polluted areas. The highest accumulation coefficient is noted for Fe and Cr.

Metals accumulation in the plants vegetative buds of the monitoring areas in comparison with the control one is observed not only in the active intrabud growth period /summer, autumn/, but in winter as well. The difference in the metal accumulation by vegetative and generative buds is not great. The maximum metal content is observed in the buds in early spring, while the minimum metal content can be noticed during the active growth period. In the experienced variants leaves the metal accumulation is actively implemented before July. Then this process becomes slower.

According to the heavy metals accumulation in the conditions of heavy metals contamination, the plants organs can be placed as follows: roots > leaves > buds > stems > seeds.

In *Acer platanoides* organs in polluted areas such metals as Cr, Fe, Pb and other heavy metals are accumulated in larger quantities than in other species of maples. Small quantities of heavy metals are accumulated in *Acer negundo* organs in the polluted environmental conditions.

The problem of use of heavy metals accumulation indices in the organs of maples for the environment pollution monitoring during different seasons is being discussed.

EFFECT OF IRON AND MANGANESE EXCESS ON GROWTH AND POTASSIUM ACCUMULATION IN ORGANS OF *ARCTOTIS STOECHADIFOLIA* BERG AND *CALENDULA OFFICINALIS* L.

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The influence of excess iron and manganese in soil near the metallurgical plant on growth and content of potassium in organs *Arctotis stoechadifolia* Berg and *Calendula officinalis* L. in different periods of vegetation was studied. It was established that contaminated soil with iron and manganese inhibited growth of vegetative and reproductive organs of plants. The quantity of shoots, leaves and area of leaves was decreased. It led to essential reduction of assimilations surface of plants.

Growth of vegetation organs *Calendula officinalis* L. was suppressed more than *Arctotis stoechadifolia*. *Arctotis stoechadifolia* was resistant species to excess of iron and manganese in soil not only by growth parameters but in visual assessments of leaves damage.

Contamination of soil with heavy metals decreased content of potassium in both species of plants, especially in leaves. There was shown that its content in generative organs buds and flowers by influence of heavy metals was decreased. Concentration of elements in stems of both species was changed less. Excess of Fe and Mn in soil strongly inhibited accumulation of potassium in organs *Calendula officinalis*.

The most differences in the level of potassium in organs of experimental plants were in stage of three leves and stage active growth of plants. These differences were less in the end of vegetation.

The coefficient of correlation ® demonstrated that in conditions of environmental pollution content of potassium correlated significantly with content of iron (R=-0.98) and manganese (R=-0.80).

The same results were received in model experiments with different concentrations of Fe^{2+} and Mn^{2+} in culture media. Excess of Fe^{2+} inhibited accumulation of potassium more than Mn^{2+}.

The possible mechanism of decrease in content of potassium in organs of plants in conditions of Fe and Mn excess in soil are discussed.

GLOBAL RADIOACTIVE TRACE ELEMENTS IN SOILS AND ECOSYSTEMS IN MEDITERRANEAN LANDSCAPES OF SOUTH RUSSIA

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Some forest and steppe ecosystems in Russia are severely polluted by radionuclides from global precipitations after Chernobyl accident, nuclear tests on Novaya Zemlya, Semipalatinsk and other areas. The main aim of this study was to identify the levels of radionuclide contaminations of the forest and steppe ecosystems after global precipitations in South Russia. The study was carried out in the broad-leaved forest of Northern Caucasus (near Novorossijsk) and in the steppe of Predcaucasus (Rostov region). The radionuclide (^{90}Sr, ^{137}Cs) concentrations in the soil, forest litter, fresh dead leaves (oak, beech, elm), soil dweller, mollusc and amphibian bodies, bones and bodies of birds and mammals were measured. We collected bones of dead birds and mammals and obtained bird bones from hunters too.

The samples of brown soil from the oak-beech forest and black earth from steppe zone were characterized by high humic concentrations and acidities similar to neutral. The ^{90}Sr budget of brown forest and black earth soils were 7.6 and 5.3 GBq/km^2, a one of ^{137}Cs - 8.2 and 12.5 GBq/km^2 relatively. In oak-beech forest the highest radionuclide concentration were found in litter. It was peculiarity during periods where global radionuclide precipitations were maximum. The property of biogeochemistry processes in black earth soil was the exit of radionuclides from litter and migration to deep layers of soil, where their accessibility for root systems may be to increase.

The molluscs (dwellers of forest litter and herb layer of vegetation) are important component of forest ecosystems. The ^{90}Sr concentrators of out molluscs were the litter organisms and the largest species *Helix albescens*. The positive correlation between coefficient of accumulation and body size of molluscs were found. ^{137}Cs concentrations in mollusc bodies and that in fresh dead leaves were similar. The highest concentration was found in the body of *Bradybaena fruticum*.

^{90}Sr concentrations in *Rana ridibunda* bodies were similar to those in mollusc bodies. In the forest and steppe ecosystems ^{90}Sr accumulation in bird and mammal bones depended on taxonomic differences, age, sex, role in food chains. species biology. In steppe ecosystem the ^{90}Sr contents in bird bones varied in large ranges. Acceding to preliminary results the predators more accumulated ^{90}Sr, than granivorous species.

The study revealed that the animals with calciferous skeleton (all vertebrates, millipedes, chelly molluscs of out invertebrates) accumulated ^{90}Sr more than other organisms. ^{90}Sr accumulation rates in these animals were higher than 1. However radionuclide concentrations in animal bodies were always lower than those in soil. These animals may be good bioindicators of ^{90}Sr contamination in prolonged monitoring of ecosystem in South Russia, but best bioindicators of ^{137}Cs contamination may be only vertebrates.

RESPONSE OF PLANT SPECIES TO CONCENTRATION LEVELS OF Zn, Pb and Cd AS INFLUENCED BY SOIL CONDITIONS

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The ability of soils to accumulate heavy metals in a top fertile layer causes many problems, which are related to the danger of consumption of toxic vegetable products by humans. The quantification of arable lands suitability for various crops implies simulation of heavy metal behaviour under various soil conditions and heavy metal concentrations.

To achieve this purpose field and laboratory studies of plant uptake and Zn, Cd, Pb mobility have been carried out with various doses of heavy metal combinations simulating artificial soil pollution. Experiments comprised major crops and soil types occurring in the European part of Russia. Namely, these were: sod-podzolic (Podzoluvisols) loamy and heavy-loamy soils with different cultivation degree and typical chernozem. Humus content ranged from 1.8 to 8.2%, pH(H_2O) - 4.5-6.3, cation exchange capacity was equal to 10.8-51.7 cmol (+)/kg. Crops were: barley, oats, vetch, green vegetables, lupin, fodder grass and edible roots.

The statistical analysis of experimental data has revealed high correlation between heavy metal concentrations in soil extracted by ammonium acetate buffer at pH 4.8 and in plants. The properties of this extractant are similar to those of root releases, therefore, the extract obtained corresponds to bioavailable forms of heavy metals in soils.

Bioavailable forms are controlled by the total amount of metal and by soil properties, the latter being: organic matter content, pH, redox, texture. Depending of these factors ammonium acetate buffer extracts 36-66% of total amount of Pb, 57-96% of Cd and 65-90% of Zn from soil samples. The effect of synergism, i.e. the increase of one metal mobility with the increase of the other metal concentration, was evident at increased doses of metals, as well as in soils with high buffer capacity of high farming.

It was found out that the uptake of heavy metals by leguminous and green crops was more intensive in comparison with cereals. In respect of heavy metal tolerance the considered species can be arranged as follows: barley and oats > fodder grass > edible roots > vetch and lupin > green vegetables.

Crop yields are known to be influenced by heavy metal concentration in soil as well. In terms of yield levels metal supplies were categorized by B.J. Alloway (1990) into three groups: insufficient, sufficient and excessive. These categories were determined in experiments with cereals; there was no yield increase caused by heavy metals for green and leguminous crops.

The obtained results have been used as information basis in computer system intended for ecological assessment and for substantiating melioration measures of croplands subjected to heavy metal immission. Heavy metal concentration and yield of crops are described by functions, individual for each plant species. As the argument we used the concentrations of heavy metals extracted by ammonium acetate buffer from the soil.
These concentrations were derived from multidimensional regression equation using total amount of metals in soil and the above-mentioned soil properties as input variables.

Set or regression models have been complemented by qualitative information in the form of sequences of crops with decreasing tolerance to each metal and of soil type. These data are applied when the required equation is missing. In this case the results of assessment will be represented by two values - upper and lower estimates derived from equations for other crops and soils.

THE GRAVITY AND THE UNIVERSALITY OF THE DEFICIENT DISORDERS AND THE INTOXICATION OF SOME TRACE ELEMENTS IN CHINA TODAY

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Because of long-term role of geographic and geochemical environment, the content and biological availability of trace elements in plants, animals and drinking water are very different in every area in China and the world. So that, the intake value and the absorbent rate of different trace elements from diets are also not alike. As a result of the phenomena mentioned above, the intake value of one or several trace elements can be more or less than the human requirement. Therefore the deficient disorders and the intoxication of some trace elements are most widespread not only in China but also in the world for present time.

The investigation result for 16 provinces in China indicated that the incidence of nutritional anemia was 52.9% and 73.7% in pre-shool children in the city and in the villages respectively, 44% in the women and 60% in pregnant. The zinc deficiency is very widespread in pre-school children in our country. Based on the research results in 20 cities, the incidence of zinc deficiency reach to 40-60%. On the other hand, 82.95 million people were threatened by environment low selenium in 310 counties of 16 provinces in our country. And many people have been suffering from Keshan disease which related to lower selenium intake. The iodine deficiency is also severity in our country, basing recent studies that perhaps 300 million people have been threatened by low iodine intake and 35 million people were suffering from Goitor. The population of many areas was threatened by low fluoride and low molybdenum in the environment.

As the deficiency of essential trace elements, the incidence of the intoxication of trace elements are severity and widespread (such as Pb, Cd, Hg, As, etc.). The essential trace element may also be toxic, if taken in excess of requirement, example 12.82 million people suffered from endemic fluoride toxication in 12 provinces in North China; if adds the value of the points and area investigation in South China, more than 45 million people were threatened by high environmental fluoride level.

THE DISCOVERY OF ORAL ZINC THERAPY AND ITS CLINIC THERAPEUTIC EXPERIENCE IN 3385 CASES OF ACNE VULGARIS AND OTHER DISEASES DURING 1959-1991.

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External use of zinc sulfate solution (eye drop) accelerating healing of chronic leg ulcers was found by author in 1958. And then, the trial of oral zinc was done on cats, dogs and rabbits. No obvious toxic effect was observed for 5-10 mg Zn^{++}/kg body weight/day in two months. The author himself, with 60 volunteers, was administrated oral $ZnSO_4$ solution in different doses (300-450 mg $ZnSO_4$ per day) during May-August, 1959. No side effects were observed. 0.5% $ZnSO_4$ solution was found better than other concentrations and was used thereafter.

A treatment with oral zinc by using above mentioned on 26 cases of chronic leg ulcer and 25 control cases of similar conditions was carried on in August, 1959. The beneficial results were observed by an accelerating healing of leg ulcer in old farm workers. This was first finding of oral zinc in human beings which was later confirmed by Prasad (1963), Pories (1967) and Hassian *et al.* (1972).

During 1959-1991, 3385 skin cases, i.e. 2000 acne vulgaris, 100 chronic eczema, 54 chronic leg ulcer, 65 neurodermatitis, 106 miscellaneous and 060 cases of children and other adult's diseases were treated with zinc sulfate orally. Usually, the dosage was 0.5% $ZnSO_4$ solution, 20 ml or 30 ml three times daily for adult and Zn^{++} 1mg/kg/day for children. The therapeutic course was 4-8 weeks. The effects appeared 3-7 days after zinc therapy in acne. There was no adverse effects. The rate of effectiveness achieved to 95% in 2000 cases of acne vulgaris, 84% in chronic leg ulcer, 88% in chronic eczema, 53% in neurodermatitis, 46% in psoriatics and 92% in children with poor appetite. However, response to psoriasis was poor. And excellent response of oral zinc preparation was obtained in most acne vulgaris patient with normal, decreased or increased serum zinc level.

It is concluded that excellent nutritional, physiological and therapeutic response of administration of zinc preparation would be used as a rational criterion for evaluating zinc nutritional condition in human beings (especially in evaluating zinc deficiency). This study recommended emphatically that the use of oral zinc treatment for individual patient must be applied with small doses (300-450mg $ZnSO_4$ per day for adults), short courses (6-8 weeks) and using very dilute concentration.

MULTIFACTOR MONISM SYNTHETICAL CONTROL THEORY --- THE RELATIONSHIP BETWEEN TRACE ELEMENTS AND AGING, DELAY OF AGING, CANCER, CONGENITAL DEFORMITY AND CARDIO-VASCULAR DISEASES

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INTRODUCTION

Aging (senescence) is a natural unavoidable law in human being as well as in any life things. But the delay of aging is also possible. In order to guarantee that human health longevity, specialists must be research further the mechanisms of aging and the means of delay of aging. For this purpose, a large numbers of aging theories have been proposed. We can see more than two hundred aging theories of different points of view in the biological and medical literature. But the author considers and believes that "free radical", "peroxidation", "compartmentalization" and author's "trace element" theories are the most foundational factors and most research future and most convining ability during the many aging theories.

Although they are alone with others, but using the point of quantum biology (submolecular biology) and trace element, the author can string successfully this four theories and advanced a new synthetic theory which can unify be explain the mechanism of aging, cancer, congenital deformity, cardio-vascular and many other serious diseases --- Multifactor Monism Synthetical Control Theory (MMSC theory or Kong's theory) established in 1975 and reported at plenary session at the First Aging Science Congress of China in 1984. And reported at plenary session by special invitation of Dallian International Gerontology Congress in 1988.

The oversea and Chinese specialists gave high evaluation for my new opinions. This theory was acclaimed by specialists as "Kong's theory" for aging, cancer and congenital deformity.

BRIEF OUTLINE OF AUTHOR'S THEORY

The main content of author's theory is that the above four factors may play physiological and pathological roles in "four in one" in the aging and the diseases mentioned above. But, trace elements especially Se, Zn, Mn, Fe, Mo, Cr, I and thier chelating compounds (in particular, the enzymes containing trace elements, for example GSH-px, SOD, POD, CAT, etc...) and leading place. Trace elements may highly be relevant to the control of the formation and the elimination (scavenge) of free radical, against the damage of peroxidation, the protection of cell membrance, the synthesis of DNA, RNA and protein, and the maintenance of the compartmentalization condition. If the deficiency of trace elements or activity decrease of the enzymes containing trace element, there will be occur an increase of free radicals, the strengthening of peroxidation, the breakdown of compartmentalization condition, and further attack and damage the DNA, RNA, proteins, enzymes, the cell membrance, cell plasma, cell nucleus and other sensitive life constituents. And induce further many biochemical disturbances and pathological changes in the metabolism process, the immune process, the structure and functions of endocrine, the cross-linkage of nucleic acid and protein, the genetic factor, even occur the mistake of gene expression and gene mutation. As a result of above harmful changes, speed-up aging of cells, tissues, organs and whole organism, and various diseases (such as cancer, congenital deformity, cardio-vascular diseases, rheumatism, etc.) will take place.

Therefore, many other theories of aging and cancer (including "Cross-linking", "Indocrine", "Immunology", "Metabolism" theories, etc.) are not guiding factors, there are all intermediate links. Our "Multifactor Monism Synthetic Theory" reflects the original foundation of etiology in submolecular level in the aging and above pathological disorders (figure 1).

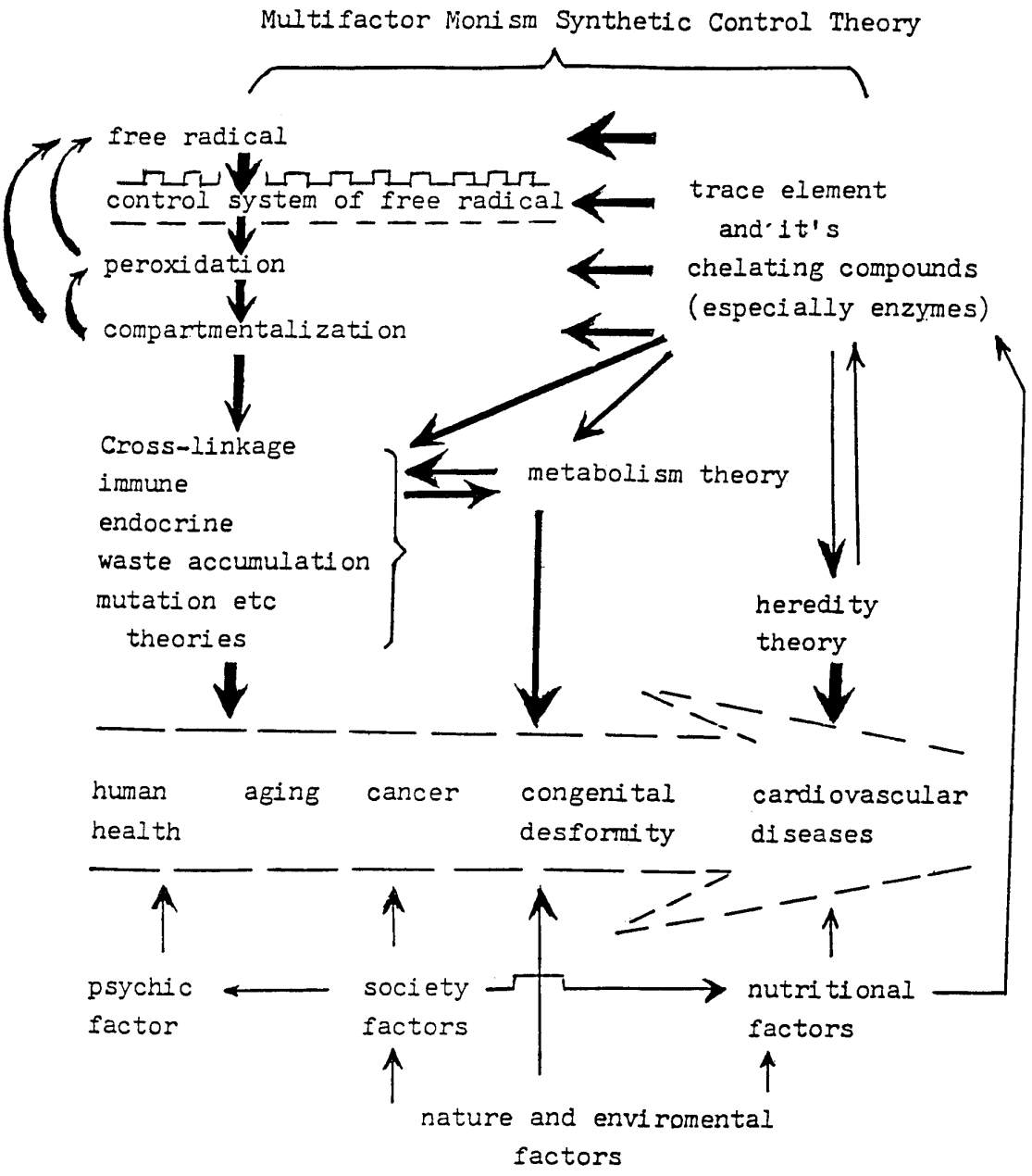

Figure 1. The principal and suborinate and supplement each orther relationship between author's Multifactor Monism Synthetic Control Theory and other aging theories.

ESSENTIAL TRACE ELEMENTS
---THE LIFE SPARK

Any materials (including the most sensitive and complicated active compositions of human brain) are constituted by the elements in all over the world.

It is generally acknowledged that the original life, pants, animal and human being all formed and evaluated from abiotic elements. But, before the occurrence of original life substance, protein and enzyme in the world, how the nucleic acid and protein synthesized from inorganic elements in the original earth? How they are linked up with each others? There are all unsolved important questions and argumentive focus in the area of the natural and the social science.

It is very worth rejoicing the Ogell's study have been confirmed the zinc ion alone can catalyze be nucleic acid duplication and may be forms 40 RNA linking pattern is similar with modern RNA on artificial synthetical RNA (polymerized-c template during the condition without the enzyme. And the duplicative miscoding rate was only 1/200 for polymerized-c in catalyzed reaction of zinc ions, the duplication miscoding rate compare with present nucleic acid enzyme is very similar.

This is an extraordinary discovery for study the source of original life, because some researches confirmed already that if imitate reducative atmosphere condition of original earth, take engaged in an artificial spark discharge, there will be form whole 20 types of amino acid which the constilution need of living beings.

On the other hand, during the synthesis and metabolism processes of nucleic acid, not only in original earth but in present mankind, zinc ion still plays an important biochemical role. Because of DNA-polymerase, RNA-polymerase, thymidine kinase and 200 kinds of enzymes are zinc-depend enzymes; zinc also regulates the activity of RNase; the catabolism of RNA appears to be zinc-depend. The effects of zinc on protein synthesis and aging may be attributable to its vital role in nucleic acid metabolism. Besides these functions of zinc mentioned above, it may be stabilization of biomembrance, against the toxic roles of Cd, Pb, Cu and can scavenge be free radical, especially in the control of cell division zinc occupy even more important place.

TRACE ELEMENTS INFLUENCE HUMAN LIFE-SPAN

Since trace elements are so important for human beings. And toxic trace elements are so harmful for human health. However, due to a long-term role of geographic and geochemical environment, the deficient disorders and the intoxication of some trace elements are most widespread and grim not only in China but also in the world for present time.

Based on an investigation of WHO, the incidence of nutritional anemia reached to 20-80% in the women in different countries. The incidence is most high in Middle East, South America, Asia and Africa, especially in low economic areas. The incidence of nutritional anemia is also higher in North America and North Europe.

The zinc deficiency is common in the world. The recommended diety allowance (RDA) for zinc is 15 mg/d for a health adult. In the Amazon, basing Shrimpton showed that the children consumed, on average 47 per cent and adults 49 per cent of the RDA for zinc.Three recent studies from USA have shown that over two thirds of the population failed to reach a daily intake of 2 mg/d of the RDA for copper. Schroeder's studies indicated that the marginal deficiency of the chromium was widespread in the USA. Iodine deficiency afflicts perhaps 200 million people in the world today. Besides these, the deficiency of fluoride is also widespread in the world and China.

The investigation result for 16 provinces in China indicated that the incidence of nutritional anemia average 52.9% and 73.7% in pre-school children in the city and in the villages respectively, 44% in the women and 60% in pregnant. The zinc deficiency is very widespread in pre-school children in our country. Based on the research results in 20 provinces and cities in our country, the incidence of zinc deficiency of pre-school children reach to 40-60%. On the other hand, 82.95 million people were threatened by environmental low selenium in 310 counties of 16 provinces in our country, and many people have been suffering from Keshan diseases which related to lower selenium intake. The iodine dificiency is also severity in our country, basing recent studies that perhaps 300 million people have been threatened by low iodine intake and 35 million people were suffering from Goitor. The population of many areas was by low fluoride and low molybdenum in the environment, which inducing the oesophagus cancer spread and high incidence in four area of China.

As the deficiency of essential trace elements, the incidence of the intoxication and environmental contamination of trace elements are severity and widespread, such as Pb, Cd, Hg, As, etc. (table 1). The essential trace element may also be toxic, if taken in excess of requirement, example 12.82 million people suffered from endemic fluorosis in 12 provinces and cities in North China; if adds the values of investigation at the different points and areas in South China, the threatened population reached to 45 million caused by high environmental fluoride level.

As mentioned above, we can see that the incidence of the deficiency and the intoxication of trace elements are highest and most grave in China and in the world. There must be disturb seriously human health, Life-Span and the longevity.

LES PARTICULARITES D'ACCUMULATION DES METAUX LOURDS DANS LES ORGANES ET DES TISSUS AMPHIBIENS EN RAISON DE CHANGEMENT DE BIOGEOCHIMIE A L'ETAT DU MILIEU NATUREL AU SUD-EST DE L'UKRAINE

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L'influence des facteurs technogènes dans l'industrie moderne à la région du Sud-Est de l'Ukraine aboutira au changement considérable de la composition biogéochimique du milieu naturel, et au premièrement, vers l'augmentation de l'eau, sol et l'atmosphère de 2,5 à 100 fois des métaux lourds par rapport aux caractères des fonds naturels exposants pour l'écosystème indique. Cela se donne pour le changement de leur niveau dans l'organisme des différents représentants de zoocénose. Et au premier rang, cohérent avec de l'eau et du sol d'« Anura Rafinesque ».

La recherche financée par le Comité des Sciences et de la Technologie de l'Etat d'Ukraine, transmettre au thème : contenu des métaux (fer ; manganèse ; cuivre ; zinc ; nickel ; plomb et cadmium) dans les organes et des tissus Amphibiens, ont montré que les animaux en grande partie accumulent des métaux lourds. Avec cela, le contenu des microéléments dans les organes et des tissus des animaux corrélatifs avec leur niveau dans l'environnement, et obtiennent 100 fois et plus. A la suite, on remarque que la plus grande partie des métaux accumulent par l'espèce d'Amphibie qui vit dans l'eau (*Rana ridibunda, Bombina bombina*), et le moindre par l'Amphibie terrestre (*Pelobates fuscus, Bufo bufo, Bufo viridis, Hyla arborea*). Avec cela, par la contenance des microéléments dans les organes et des tissus Amphibiens, on peut suivre la voie de leur admission dans l'organisme et leur extremination.

Alors l'admission des éléments toxiques : plomb et cadmium, en premier lieu, lié avec l'atmosphère pollué, en quoi témoigne de leur contenance dans les poumons, et l'extremination passe par les rognons, dans ce moment les autres éléments comme : fer, manganèse, cuivre, zinc, nickel passent par la peau et l'estomac (avec l'aliment) et rentrent dans l'organisme, en quoi parle leur niveau élevé dans ces organes et tissus, en même temps leur extremination aussi passe par les rognons. On souligne que le niveau élevé contenu tels éléments comme cadmium et plomb dans les organes et des tissus des animaux de biotope, où l'industrie n'existe pas. A la suite supposons que, cet lié avec leur admission à l'environnement avec les poisons chimiques et pesticides, utilisant à l'agriculture.

Les organes et des tissus des animaux, qui habitent aux biotopes à la place de traitement de manganèse, des fers-minéraux et au lieu de butin on remarque que l'accumulation considérable de manganèse et de fer, et aussi leur accompagnant par nickel, à ce moment-là comme aux animaux des biotopes submersible de réservoir du Zaporogie passe à l'accumulation considérable de : fer, zinc, cuivre et de nickel.

Ainsi, l'analyse de l'accumulation des métaux différents dans les organes et des tissus des espèces différentes d'Amphibie a montré que leur niveau en grande partie lié avec l'état du milieu habitat, qu'on peut être utiliser comme dans le système biomonitoring et aussi pour le pronostic de l'état zoocénose auprès de l'augmentation technogène de l'environnement.

ECOLOGICAL AFTER-EFFECTS AND ^{90}Sr DYNAMICS IN SOILS OF SOUTH URAL RADIOACTIVITY POLLUTED AREA (1957-1994)

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The ecological after-effects of soil pollution by ^{90}Sr and radionuclid biological migration were studied in field experiments using soil animals as bio-indicator of radioactive pollution in South Ural area after "Kyshthym accident" 1957. The radioactive soil pollution exarts the area impact on the permanent soil dwelling animals. As a direct effect it has been seen the appreciable reduction of population density, disturbance of the breeding process, loss of the biological diversity at the polluted soil areas. In fact a soil with high level of ^{90}Sr and a radiation level above 1-3 R/day contained 10-folg reduction of soil inhabiting millipedes, earthworms, insect larvae, enchytraeidae. In 1968-1994 the secondary side-effects have been registrated for active migrants among terrestrial animals: birds, mammals, insects. The accumulation of ^{90}Sr in soil animals, mammals, birds, reptiles, insects and plants was studied, as a radionuclide migration in polluted ecosystem.

LA COMPARAISON DE CARACTERISTIQUE DE L'ACCUMULATION DU CADMIUM ET DU PLOMB DANS LES ORGANES ET DES TISSUS DES DIFFERENTES ESPECES DE L'AMPHIBIE AU TERRITOIRE DES RESERVES DU PRIDNEPROVIA

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Actuellement dans le résultat de toute la production élargie s'observe la saturation progressive des métaux lourds aux biosphères. A part de préoccupation rappelle de ce fait qu'il est dans le processus technogène des métaux passent à l'état diffuse en grand actif.

Pour la compréhension correcte des évènements processus et leur conséquences nécessaires de la recherche comme de fond naturel contenu des microéléments dans les organismes vivants et ainsi leur accumulation dans les organismes des animaux aux conditions de l'influence technogène. A part de l'intérêt rappelle l'accumulation des éléments toxiques comme le cadmium et du plomb dans les organes et des tissus des animaux de biotope « sous condition propre » comme au zone de la réserve du Dnepropetrovsk-Orel.

La détermination de ces éléments dans les organes et des tissus de l'Amphibie des biotopes de la réserve naturelle, du Dnepropetrovsk-Orel a montré leur existence dans tous les organes et des tissus de l'Amphibie.

La plus grande partie de ces éléments toxiques contiennent dans les organes et des tissus de « Rana ridibunda ».

Le contenu du plomb dans les rognons de cette espèce obtient 666.69 mg/kg de masse mouille, dans ce temps-là, le niveau du cadmium un peu bas, et dans les poumons masse mouille obtient maximum 397.86 mg/kg.

Le contenu du cadmium tout entier dans tous les organes et des tissus d'Amphibie est plus bas par rapport au plomb.

La comparaison de l'analyse du plomb et du cadmium contenu dans les organes et des tissus des différentes espèces d'« Anura Rafinesque » a montré que la décroissance de degré de leur contenu dans la plupart des organes et des tissus de l'espèce d'Amphibie examinés on peut mettre en ordre ci-dessous : *Rana ridibunda, Rana arvalis, Bombina bombina, Hyla arborea, Pelobates fuscus.*

Avec cela, si l'accumulation du plomb pour le principal, remarque dans les rognons et les poumons, alors l'accumulation du cadmium le plus variable. Le niveau maximum du cadmium établi dans les poumons de *Rana ridibunda* et de *Rana arvalis* ; *Bombina bombina* - dans l'intestin et les rognons, et pour *Pelobates fuscus* et *Hyla arborea* - dans les rognons et le foie.

Ainsi donc, le contenu de coefficient maximum comme le plomb, et comme le cadmium, remarquent dans les organes des différent niveau élevé du métabolisme: rognons, le foie, les poumons, et les exécuteurs d'une fonction précise dans l'organisme : respiration, et

l'extremination: les poumons, les rognons, l'intestin, qui donne la possiblité précisée de la voie d'admission du detoxique et l'extremination des éléments toxiques de l'organisme.

A la suite, on remarque l'extrêmement de ce fait positif de l'accumulation du cadmium et du plomb dans les gonades de toutes les espèces d'Amphibie qui doit être donnes le processus de la reproduction des spermioles évolues et des lavres.

La plus faible concentration du cadmium et du plomb fixe dans les tissus musculaires d'Amphibie.

Le résultat obtenu permet parler l'admission des éléments toxiques: plomb et cadmium dans l'organisme d'Amphibie qui viennent de l'air atmosphérique et passe par les poumons, dans ce moment-là, le contenu de ces éléments dans l'estomac qui entre avec les aliments, et qui viennent du milieu d'eau entre dans la peau, par rapport aux autres organes et tissus bas.

A la suite supposons que l'encrassement du zoocenose tout entier au territoire du Dneprovsko-Orel de la réserve, pour le principal passe par l'atmosphère avec le flux d'air, jeté par l'industrie.

HEAVY METALS IN VERTEBRATE ANIMALS IN AIR POLLUTION ZONE AT KOLA PENINSULA

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The complex researchs of pollution influence on land ecosystems were conducted in 1991-1992 in Murmansk region in the zone of Pechenga-Nikel plant influence. The most numerous animal species inhabiting this territory, which do not migrate on long distances and are of different trophic levels, were chosen as bioindicators of heavy metal pollution of the territory and concentration in organisms of vertebrate animals. Among them there were mammals - mice (*Microtus oeconomus*), grey-sided voles (*Clethrionomys rufocanus*), common shrews (*Sorex araneus*), birds -willow ptarmigan (*Lagopus lagopus*).

The sample squares were chosen according to the air basing pollution maps, the researchs of solid dust quantities and the levels of plant degradation. Those squares were situated to the south from the polluter at the distance of 5-6, 8-10, 20, 30 and 40 km and were ranged according to the quantity of solid particles on the square units. The sample square at 40 km distance was taken as the background level of pollution.

Small mammals were caught at all sample squares with the help of break-back traps according to the standard methods. Two hundred traps were put into two lines and left for two days in autonomic and accumulative positions of the landscape in natural biotops. We made morphometric measurements from catched animals and took the following organs and tissues for chemical analysis: from mammals - liver, muscles with bones and skin, from birds - only liver. Eight chemical elements (Ni, Cu, Zn, Fe, Pb, Cd, Co, Cr) were analyzed in organs and tissues of vertebrate animals. All analetic works were conducted according to the standard methods, heavy metals were determined on the atomic-absorbtion spectrometer with further mathematical analyses of the results with the help of computer programs.

Our researchs showed that for all investigated species high heavy metal concentrations were marked close to the smelter (5-6 km to the south). However, it was shown a rapid decrease in metal levels when the distances from the smelter increased. For example the concentrations of Ni, Cu, Zn, and Fe were 4-6 times higher at 5-6 km distance compared to 30 km from the smelter. From the other point of view heavy metals concentrated mainly in liver and skin of the animals. The character of heavy metal concentrations in organs and tissues of 3 mammal species appeared to be quite different. Those species differed in concentration of Fe, Cu and Zn in liver, Cr, Ni and Pb in bone-muscle tissues, Cr, Ni, Cu and Cd in skin.

The main effect of the smelter on wild living vertebrates seems to be reduction (and changes) in population densities for birds and mammals caused by the habitat changes. Dramatic changes in the areas where all or the most of the vegetation was dead (approx. 800 km^2) and diffuse effects in the areas where vegetation changes had occurred (approx. 5 000 km^2).

Our and literature data showed that the manner of animal life and the character of trophic activity play the main role in heavy metal concentration in their organisms. Because of that nevertheless the dependence of heavy metal concentrations in animal organs and tissues while moving off the smelter is the same as in plants and soils the levels of those concentrations are quite different.

ASPECTS OF REPTILES RESPONSE TO HEAVY METALS CONTAMINATION: SPECIES DIVERSITY, BIOACCUMULATION AND BIOCHEMICAL ALTERATIONS

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Environmental pollution in Ukraine had reached so scale that the Parliament declared Ukraine as a zone of environmental disaster. Dnepropetrovsk region is mostly industrialized in the state. Tens of chemical, metallurgical and metal-working plants with insufficiently clean technologies turned the most part of the region into a "firing ground" of soils contamination. This strong pollution has inevitably to lead to disturbance of the vital activity of biosystems: from cells to ecosystems. Herpetofauna is an important functional constituent of ecosystems, which sustains their biodiversity and stability. But, until recent times, reptiles were not practically investigated from the viewpoint of soil contamination as opposed to small mammals and soil invertebrates. And they have not gained widespread acceptance as indicators of pollution.

Our researches were carried out in the vicinity of chemical and metallurgical plants of Dneprodzerzhinsk (city in the Dnepropetrovsk region). For various estimates these plants throw out from some to tens of hundreds tons of heavy metals annually. Relatively pure biotopes of the Dneprovsko-Orelsky Nature Reserve were as a control. Investigated biotopes in the polluted zone and in the Reserve are of the same ecological and soil type.

According to our data it is observed the reduction of species diversity of reptiles in the contaminated zone. Only two species - the grass snake *Natrix natrix* and the sand lizard *Lacerta agilis* - were found here as distinct from the Reserve where six reptiles species were registered. The reduction of the reptiles species diversity accounts for 66.7%. The lizards and snakes populations are oppressed in the polluted biotopes. Their abundance is less than in the Reserve by a factor of 21-30.

We studied iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), nickel (Ni), lead (Pb) and cadmium (Cd) bioaccumulation in liver, kidneys, skin and bones of the grass snake. It was used the atomic-absorption spectrophotometry method with AAS-30 (Karl Ceys, Yena, Germany). Our researches showed the considerable heavy metals accumulation in the snakes from the contaminated zone except Zn content in the liver. It was more for the Reserve - 47.7 mg/kg of dry weight on the average while the Zn content in the snakes liver from the polluted biotopes was 30.4 mg/kg dw. Apparently, it may be connected with the high level of Cd in the snakes liver from here - 3.9 times more than in the Reserve - and Cd is known to be a strong antagonist of Zn. In all tissues investigated the most pronounced increase was determined for toxic metals: Pb and Cd. So, Pb content in the snakes liver from contaminated zone was 4.3 times more (23.4 mg/kg of dry weight on the average), in kidneys - 3.0 times (78.2 mg/kg dw), skin - 2.4 times (15.9 mg/kg dw) and bones - 4.1 times more (84.9 mg/kg dw) than in the snakes from the Reserve Cd content increasing was further pronounced : 3.9 , 5.4 , 5.1 and 13 (!) times respectively. Bioaccumulation dynamics of heavy metals among the organs under investigation had been changed as well. Concentrations factors (ratios of tissue and soil concentrations), calculated for all metals assayed, ranged variously. It was less than 1 for Fe but averaged 100 for Pb and Cd.

Taking into account that the content of total protein and lipids reflects state of constructive and destructive metabolism we have analyzed protein and lipids levels in liver, kidneys, heart, lungs, skin and muscular tissue. Data obtained testify that the considerable alterations of these indices in the contaminated biotopes exist. So, in the polluted zone decreasing protein content in the snake liver (from 12.6 to 10.4% of fresh weight) keeps pace with the lipids content increasing (1.7 times) as compared with the snakes in the Reserve. Obviously, it may be a result of the metabolism disturbance brought on by the toxic effect attending with the liver damage. It is necessary to mark that protein increasing was observed in the snakes skin (from 21.6 in the Reserve to 29.2% in the polluted zone) and kidneys (from 7.2 to 9.6%). It seems likely that kidneys put out heavy metals and they do so excreting the metals with the protein complexes. As to skin, it may be safely suggested that metals accumulation must be accompanied by metal-protein complexes synthesis which can entail darkening reptiles color (Sharygin, 1985). Form the result obtained it may be deduced that in the polluted zone the lipids content increases in all "quiescent" organs and decreases in "muscular" organs: heart and muscles.

Thus, the considerable alterations in protein and lipid metabolism, a high degree of heavy metals bioaccumulation in the snakes body under conditions of industrial pollution bear witness to the possibility of reptiles using in bioindication.

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HEAVY METALS ACCUMULATION IN MOUSE-LIKE RODENTS FROM HABITATS OF DIFFERENT TRANSFORMATION RATES

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Ecotoxicology of small mammals attracts a great attention because determining adaptation mechanisms of animals discovers possibilities of human adaptation to increasing environmental pollution.

The investigations of mouse-like rodent *Apodemis flavicollis* performed in Dneprovsko-Orelsky reserve and in the biotopes affected by sewage waters, chemical plants air exhausts showed that the process of both biogenous (iron, manganese, copper, zinc) and toxic metals (lead, cadmium) accumulation in tissues and organs took place. In the animals from the industrial pollution zone the great lead and cadmium accumulation was found in the lungs, stomach and kidney. It was 1.5, 1.3 and 3.1 times more for the lead and 2.3, 6.5 and 1.3 more for the cadmium in these organs respectively as compared with the reserve. The content of iron, manganese, copper, zinc and others in these organs is more than MAC by a factor of 2-3.

In decreasing order of iron content the organs ranked as follows: stomach, lungs, intestines, liver, kidneys, fur, bones and muscles. The manganese and copper content is maximum in lungs, the zinc - in kidneys. Besides that, a considerable number of copper was in stomach and kidneys, of zinc - in liver, stomack and intestines. Apparently, it is connected with the entering of trace elements with a food. The most quantity of nickel was in the lungs of animals. It is necessary to mark that the lungs contain a much number of all investigated elements (except zinc). It is connected with a high degree of air pollution.

Thus, under conditions of environmental pollution mouse-like rodents are good indicators of environmental pollution by both biogenous and toxic heavy metals. It makes possible to do a prognosis of state of mammals populations under anthropogenic pressure and to determine of the metabolism disturbance.

TRACE ELEMENTS MIGRATION IN SOIL-HERBACEOUS PLANT SYSTEM UNDER ANIMALS FOSSORIAL ACTIVITY

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It was investigated iron (Fe), manganese (Mn) and zinc (Zn) accumulation in the birthwort *Aristolochia clematilis*, the ground ivy *Glechonia hederacea*, the celandine *Chelidonium majus*, the starwort *Stellaria holostea*, the milfoil *Achillea milefolium*, the violet *Viola suavis*, the great nettle *Urtica dioica* in sites of fossorial activity of mouselike rodents, Eurasian badgers *Meles meles* and common moles *Talpa europaea*. Our data showed that under fossorial activity of mouselike rodents and common moles Fe, Mn and Zn content in birthwort and ground ivy decreased 1.2-1.5 times. As this took place Fe and Mn content increased in the celandine, the starwort, the milfoil and the violet as compared to control. Fe and Mn are mostly accumulated in the caulis and the leaves while Zn - in the root. But some variations of these indices were observed.

Under the Eurasian badger fossorial activity considerable variability for the trace elements accumulation was observed in the root and caulis. It is necessary to mark that under conditions of mammals fossorial activity the considerable distinctions in degree of the trace elements accumulation by the plants in relation to mechanical composition of soils. The most pronounced influence of fossorial activity on trace elements content was in the oak-forest with ash-trees of the flood land.

Data obtained as a whole showed a high degree of mammals influence on the biological cycle of trace elements in forest biogeocenoses.

EFFECTS OF TECHNOGENIC TRACE ELEMENTS ON TERRESTRIAL ECOSYSTEMS OF THE SOUTHEAST UKRAINE

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Atmospheric pollution of trace elements (Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, Al, Ca, Ag, Mo, K, Na, F) from various industrial sources has been studied for its effect on terrestrial ecosystems. The investigation were conducted at 46 sites in Dnepropetrovsk region in the South-East of the Ukraine that had different levels of industrial pollution. The control area was chosen to be similar to the study areas, and was free of industrial pollution of any type. Robinia pseudacacia (L.) was a pollutant guide plant. The soils were ordinary chernozyoms, which represent the more important soil type of this region.

The purpose of this research was to determine concentrations of 17 elements in plants (leaves and seeds) and soils and to determine any quantitative relationships between levels of these agents and state of the terrestrial ecosystems. We have studied the effects of sampling areas, difference between plant and soil accumulation levels, and the statistical contribution of the harmful components into the change for the worse of nature.

Damage criteria such as the frequency of chromosome aberrations in root tips of plants, determination of germination percentage, biomass of 1000 seeds, chlorophyll content, peroxidase activity of leaves and soils, ash of soils and plants and quality and quantity of microfauna were studied.

It was found that the concentrations of F in soils were in average 10, Zn and Pb - 3-5, Na - 2.2, Cr, Mn, Fe, Co, Ni, Cu, Cd - 2 times higher than in the control. The contents of F, Fe, Al in plants growing in polluted areas were 7, Ni - 2 Cr, Cu - 1.5, Zn, Cd, Pb, Co - 1.3 times higher than unpolluted ones. There were differences between the spectrum of plant and soil contamination.

The interest in environmental mutagenesis has strengthened considerably following understanding of the broad overlap between mutagens and carcinogens. Also alteration in environmental mutagenicity lead to increases in the mutability of living organisms. The mutation rates in the meristematic cells of plants in zones of chronic industrial pollution were 2-30 times higher than the spontaneous background level of the mutations.

It has also been shown that germination percentage, chlorophyll content, peroxidase activity of soils quality and quantity of soil microfauna were decreased. But on the other hand peroxidase activity of leaves, ash of soils and plants and biomass of 1000 seeds were increased.

A strong positive correlation was found between the concentrations of F, Fe, Cu, Zn and the upsetting of terrestrial ecosystem state.

Consequently, we have determined that among the environmental pollutants studied F, Fe, Cu and Z, were the most damaging.

SOIL INVERTEBRATES IN RISK OF CONTAMINATED SOILS ASSESSMENT

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Many potentially pollutants are released to the environment every year. Although chemical analyses of soil, air and water can provide information on the concentrations of chemicals, these analyses alone are inadequate to assess the potential toxicity to humans and wildlife. Invertebrates in these environments integrate contaminant exposure temporally and across media. Therefore, they are uniquely realistic indicators of toxic exposure in soil.

Soil invertebrate communities, their numerosity, biodiversity species composition, ecological groups and food webs understanding of heavy metals influence on sylvatic ecosystems in Moscow region. Also it was researched out the processes of accumulation Pb, Cd, Cu, Zn, by soil and soil animals. The highest concentrations of Pb and Cd was found in Aranea, Carabidae (*Calathus micropherus*), Lumbricidae (*Nicodrilus calliginosus*) - the coefficient of accumulation varies from 1.2 to 22.0, caused by habitat conditions. It was indicated decreasing of biodiversity and mesofauna numerosity on wasted plots (in 4.9 - 5.0 times). The most sensibility to heavy metals pollutions show Aranea, Myriapoda, Carabidae, Staphylinidae, Lumbricidae. The important characteristic consists in increasing the numerosity of phytophagans near autoroads. This research discovered that in roadside ecosystems the significant transformings of biocenoses occur reflecting in increasing of phytophagans numerosity and connected with it dropping of numerosity of predators and saprophytes.

HEAVY METALS IN POLLUTED INVERTEBRATE COMMUNITIES

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Invertebrates were collected by sweep net and pitfall traps in 1992-1993 in differently polluted water and terrestrial ecosystems in European Russia, Belgium and the Netherlands. Concentrations of Cu, Zn, Pb and Cd were detected in pooled samples (totally more than 1500) by flame and graphite-furnace AAS followed standard methods of sample preparations. In general the ability to accumulate heavy metals followed the order: annelids (earthworms), crustaceans (amphipods and isopods), spiders, hemimetabolic insects, holometabolic insects. West-European isopods from similar locations contained 3 times more Cu and 3 times less Zn than Russian samples. Belgian spiders contained twice more Cu, and 1.4 times less Zn than Russian samples. Contents of Pb and Cd did not differ significantly in studied groups but in comparable roadside ecosystems Dutch carabids accumulated much more Pb, than Russian carabids and spiders contained much more Pb, with comparison to ecosystems with other sources of chemical pollution. The expressed tendency of crustaceans to store copper in the row of terrestriality from water amphipods to terrestrial isopods was observed. Among grass- and soil-dwelling insects the best accumulators were saprophagans and chewing phytophagans, the worst- non-specialised predators. In putative trophic chains in Russian carabids as non-specialised predators accumulated less Cu and Zn than specialised predators, and less than specialised parasites. The expressed species-specific differences in heavy metal accumulation were found in spiders and crustaceans, while no differences were found in carabids between species, zoophagous and myxophagous species and sexes. The obtained results are discussed in relation to the problems of bioindication of trace elements in ecosystems.

L'ACCUMULATION DES METAUX LOURDS DANS LES ORGANES ET DES TISSUS D'*EREMIAS ARGUTA* AUX RESERVES DU TERRITOIRE DE PRIDNEPROVIA

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L'un de l'espèce rare de reptile: l'espèce d'*Eremias arguta* qui habite au territoire de Dneprovsk-Orel de réserve naturelle à la région de Pridneprovsk, leurs biotopes constituent au zone « sous condition propre ». A la suite, on remarque que l'adhérence de l'industrie en grand parti à la réserve échangent l'état pur du milieu naturel, comme la région de Dneprovsk entier, et ainsi, qu'il est important surtout au territoire de la réserve.

Premièrement, cela concerne l'augmentation de milieu naturel composé à la réserve, qui contiennent des métaux lourds, le milieu dans lesquels en particulier négatif, on peut être causer de l'augmentation dans les organes et des tissus des animaux toxiquent par le chrome, le plomb et le cadmium.

Les analyses concernent ces éléments entier dans l'organisme d'*Eremias arguta*, ont montré que la quantité de tels éléments comme : le chrome, le plomb et le cadmium considérablement s'augmenter dans l'organisme de l'individu à la puberté, le plus âgés du groupe, à la différence du niveau de fer et de nickel, le niveau maximal qu'on avait établi aux animaux imberbes, qui doivent témoigner à l'accumulation des éléments toxiques dans l'organisme des animaux selon leur taille.

La contenance des éléments biogènes : manganèse et cuivre se trouve au même niveau chez les individus à la puberté et à l'imberbe.

L'analyse de la recherche des éléments contenus dans les organes et des tissus d'*Eremias arguta*, a montré que la petite partie des éléments cherchés contenir dans la peau et des tissus musculaires des animaux, à ce moment-là, comme leur quantités dans le coeur, les poumons et le foie sont très beaucoup. La comparaison des contenances des éléments cherchés dans les poumons et dans l'estomac des animaux montre l'augmentation importante de leur quantité dans les tissus pulmonaires, par rapport à l'estomac. Cette augmentation constitue de 2,2 fois pour le chrome, le plomb et le cadmium - 1,9 fois, et pour les restes varient de 1,2 à 1,6 fois.

Les résultats obtenus permettent de supposer que les territoires des réserves polluées des métaux lourds se passe, principalement, par les poumons cause par l'air atmosphérique pollué par l'industrie mais ce n'est pas par objets nutritifs rentrent dans l'estomac.

L'intérêt important provoque un profond contenu des éléments cherchés dans le coeur, qu'évidemment d'une partie lié avec leur rentrés dans cet organe composé du sang des animaux, et d'autre part un niveau élevé de métabolisme de cet organe, ce qui exige ces concentrations supplémentaires des éléments biogènes: fer, manganèse, cuivre, zinc, participant aux processus de fermentation.

FLOW OF HEAVY METALS IN THE SOIL-PLANT-ANIMAL SYSTEM WITHIN A HIGH POLLUTED AREA FROM ROMANIA

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Introduction

An important and old (over 50 years) source of soil pollution with heavy metals and sulphur oxides which affects about 180,000 ha [1] in Romania in the Non-Ferrous Metallurgical Enterprise in Copsa Mica town. This paper is presenting the loading of pasture soil and vegetation with heavy metals (Cd, Pb, Zn and Cu) and their effects on animals, especially cattle, in the affected area.

Materials and Methods

The investigations were carried out within six pastures located on both slopes of the Tarnava Mare Valley including the border of the Blaj Plateau, 1.5 - 10 km far from the source, both eastwards and westwards along the main winds. On the upper part of slopes there are Regosols (loam in solum and loamy-sand in subsoil, pH 7.72-8.05, 0.8-7.9% $CaCO_3$, moderate content of organic matter), and on the rest of land there are Argilluvial Brown Soils (sandy-loam in solum and loam in subsoil, pH 5.3-6.21, low content of organic matter). The soil nutrient supply is low (N), low and very low (mobile P) and low-high (mobile K). Excepting the nearest pasture which is severely degraded and where *Agropyron repens* prevails, the other pastures have a moderate nutritive value and are covered by floristic associations of *Festuca rubra*, *Poa pratensis*, *Agrostis tenuis* and *Koeleria macrantha*. Within each pasture, 4-8 composite soil testing samples (0-18 cm depth) and plant samples (composite or individual species) have been collected.

The heavy metals in soil have been determined by extraction in a HNO_3+$HClO_4$ mixture (total forms) and in EDTA-$CH_3VCOONH_4$, pH 7.0 (mobile forms), and their fractioning according to McLaren and Grawford procedure modified [2]. The heavy metals in plants have been determined in hydrochloric solution obtained by dissolving the ash got at 450°C. Haematological and biochemical analyses, including heavy metals, in blood have been made as well as analyses of organs and tissues from normally slaughtered cattle. The atomic absorption spectrometry has been used for determination of heavy metals.

Results and Discussions

The investigations have detected the loading of soils higher than the maximum allowable limits (MAL) with total forms of Cd, Pb and Zn up to 2.3, 1.7 and 1.8 times, respectively, and with soluble forms up to 3.5, 10.5 and 3.8 times, respectively. The Cu concentration in soil is lower than MAL, but on average almost twice higher than its normal soil average content (20 $mg.kg^{-1}$) and this only on the nearest pasture (1.5 km far).

The passive uptake due to the high mobility of heavy metals in soil and the particulates fallen on leaves contributed to a great loading of grasses with heavy metals up to 14.7 times (Cd), 16.8 times (Pb) and 3.4 times (Zn) higher than the normal concentration range in pasture grasses. The correlation ratios established between heavy metal contents in grasses and in soils (soluble forms) are: 0.632 (Cd), 0.510 (Cu), 0.693 (Pb) and 0.614 (Zn) that emphasizes their

translocation from soil to plants. The high content of heavy metals in grasses decreases their nutrition value.

Besides the grasses, the grazing animals may also ingest soil up to 454 kg/cow/year [3]. Hence, the ingest of only a quarter of this amount of polluted soil in this area could daily supply 53 mg Pb and 2.1 mg Cd per cow.

Really, the grazing on such pastures has caused many cases of cattle saturnism with nervous symptomatology and mortality. For example, from 1985 to 1990, 33 lethal cases of saturnism have been recorded, and from 1987 to 1992 the following health disorders have been recorded: pregnant toxicosis, marasmus consecutive to toxico-deficient syndrome with the calves, gastroenteritis and abortion with distrophic and haemorrhagic lesions in the parenchymatous organs of abortion.

The paraclinical investigations on cattle blood show the chronic cumulative intoxication with heavy metals, especially Pb, and the metabolic profile emphasizes the following health disorders: advanced aplastic anaemia of hypochrome, mycrocitare or normocytare type as an effect of loading with Pb, Cd and Mo; leucopenia and lymphopenia; transaminase activity increase; hypocholesterolaemia; severe hyperazotaemia; and hypomagnesiaemia. On average, the heavy metal contents in the organs and tissues of normally slaughtered cattle in the polluted area are equal or higher than the MAL for Cd, while the Pb content is higher than MAL in spleen, liver, kidney and lung, and the Zn content is higher than MAL only in kidney, liver and spleen.

While there is some Cu loading of soil and grasses, a secondary Cu deficiency occurs due to the antagonism caused by the loading with Zn, Cd, Mo and Fe of animal body. Thus, the Cu content in blood is 30% lower and in liver 34 times lower as compared with the normal content.

Conclusions

This affected area is a "hot spot area", of industrial pollution type, indeed, where the chronic cumulative toxicity, mainly due to Pb and Cd, induces the main cattle health disorders. The industrial pollution identified within this area is really a severe problem whose solution needs a comprehensive approach of investigations, including a specific environmental quality monitoring system and a particular state policy aiming at the decrease of industrial emissions and the rehabilitation of polluted terrestrial ecosystems.

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ANOMALIES OF MICROELEMENTS IN SOILS AS INDICATORS OF TECHNOGENIC POLLUTION FOR URBAN TERRITORIES

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Urban areas pollution by industrial and municipal wastes accounts for concentration of diverse microelements (ME) - Pb, Zn, Cu, Cr, Ni, Cd, Hg, Sn and oth.). Elements are dissipiated by atmospheric fluxes. ME of waste waters are redistributed by the surface run off. All these fluxes are identified by technogenic anomalies in various landscape components. Investigations of the urbanized landscapes around Moscow were carried out in 1977-1990 by the Institute of Mineralogy and Geochemistry of Rare Elements (Sayet *et al.*, 1982, 1990; Sorokina, 1982, 1989); they revealed that the aerotechnogenic polyelemental geochemical anomalies in soils and bottom sediments are indicative of the technogenic pressure force on the environment and of the chemical composition of environment pollution.

The technogenic dust being incorporated into atmospheric fluxes, comes to the soil surface with the rainfall and enriches the surficial layers in microelements. In sod-podzolic soils zone on flat weakly dissected interfluves around the enterprises continuous aerotechnogenic geochemical anomalies evolve, they indicate distinctly the zone of emissions impact; their usual pattern (maximum concentrations near the emission source and their decrease towards peripheries) is in accordance with the atmospheric input. Long-term data reliably testifies to the strongest polluting effect on the humus horizon, which is known to be a conspicuous biogeochemical sorptive barrier being in direct contact with the lowermost atmospheric layers.

Anomalies in the upper soil horizon are easily recognized by the changes in total contents of pollutants in comparison with the background.

The aerotechnogenic anomaly is a polyelemental dispersion area with its centre in the pollution source. Basic ME-admixtures are stored in soils, with a distinct maximum in the centre of anomaly. All the ME occur in the centre, and only 1-2 anomalous ME may be found in its periphery. These tendencies correspond to main trends of atmospheric fall-out pattern. The following mean concentrations of ME have been observed in soils in areas adjacent to intensive pollution sources: (background concentrations are given in brackets, in mln5-10): Pb 500(25); Zn 1000(50); Cu 500(27); F 6000(210); Cr 500(45); Hg 2(0.01); Ni 500(20). So, the concentrations of main pollutants are higher than the background by 20-30 times, while in some sampling sites they may be hundreds and even thousands times higher. The peripheral parts of dispersion areas usually coalesce in modern towns, and an anomalous, spatially heterogeneous field is being formed in upper soil horizons. It comprises, however, individual local pollution nuclei, their areas ranging from 3-5 to 100-150 km^2. Integrated anomalies occur in the majority of towns with big enterprises and sprawl out of the towns; a continuous complex contamination zone is formed, its centre corresponds to the basic industrial sources.

Urban soils of Moscow district are contaminated by lead and zinc everywhere; Cu, Sn, Ag are frequent contaminants as well. Accumulation of other ME depends on the enterprises predominating in towns. Thus, with machine building and metal processing (Podol'sk, Kolomna, Dmitrov) the association of ME stored comprises Pb, Zn, Cu, Sn, Ag, W, Hg. Steel melting complexes (town of Electrostal') are surrounded by a complex anomaly with Ni, Co, Cr, W, Mo prevailing. Chemical industry and concrete production result in F, Sr, P, Hg, Ag predominance in the anomaly (Voskresensk).

When analyzing technogenic pollution in urban areas basing on studies of geochemical anomalies in soils, the migration capacity of ME - ingredients of the anomaly is of paramount importance. The specific features of sod-podzolic soils in this aspect is immobilization of some

ME on the strong sorptive barrier - humus horizon (lead, copper), whereas other elements (nickel, molybdenum) are leached due to pronounced percolative regime proper to these soils, and get accumulated in the lower part of the soil profile - radial geochemical barrier. They may also be accumulated in the final members of landscape-geochemical catenas - lateral geochemical barriers. Thus, in rivers and stream flood plains within the towns area there are anomalies originated due to both aerial and water technogenic fluxes. Studies of such anomalies should be carried out separately from those on flat interfluve surfaces.

BIOGEOCHEMICAL ASSESSMENT OF URBAN ECOSYSTEMS POLLUTION BY TOXIC MICROELEMENTS (CASE STUDIES FOR MOCOW AND NOVGOROD)

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Among the present-day fundamental geographical problems of high importance is the expert assessment of the environment status. The biogeochemical analysis of urban landscapes is regarded as its essential ingredient.

The problem of degrading environmental conditions in large industrial agglomerations of Russia became recently of great concern because of drastic adverse changes in public health. Landscape-geochemical investigations of Novgorod and Moscow, carried out by the Department of landscape geochemistry and soil geography of the faculty of Geography, comprised sampling of urban soils, archeological layers, snow, and of small streams bottom sediment; 15 microelements (ME) have been determined in the samples, many ME refer to I and II classes of ecological danger. For quantifying bulk concentrations spectral-emission method was used, mobile forms were extracted by the hydrochloric acid.

Physico-geographic and landscape-geochemical situations, accounting for the technogenic pollution by ME, have been considered thoroughly in terms of soil resilience. Thus, unfavourable environmental conditions (low evaporation, bad drainage, heavy textured sediments) in Novgorod are responsible for high pollution risk and low resilience despite moderate technogenic pressure. An opposite situation is possible as well: "geochemically favourable landscapes" (some Moscow quarters) may get transformed into ecologically dangerous ones even with moderate technogenic target, if the initial (natural) background has marginal vulnerability parameters for one or more ME.

Very dangerous are elevated concentrations of mobile forms of ME in soils (40-60% of their total content), providing for participation of toxic ME in trophic chains, with the human beings as final members. ME in urban soils are fixed at organo-sorptional and alkaline geochemical barriers.

The complex concentration index - totals of the differences exceeding the standard level, has been used as integrated criterion for pollution assessment. A series of maps has been compiled presenting concentration patterns of individual toxicants, of total pollution and of risks for soils and other environment components.

Special monitoring ecologic-geochemical maps have been proposed and compiled on the basis of original landscape-geochemical maps, maps of technogenic targets (modules of anthropogenic pressure) and information on the functional land use of the urban areas. These maps serve a basis for regional landscape-geochemical forecasts of urban environment status in the next dozen years. Five gradations to characterize the ecosystems status have been chosen for the legend of such maps: normal, unfavourable, dangerous, critical and catastrophic.

The urban environment is assessed as unfavourable or even dangerous over the majority of urban areas studied; higher risks (critical and catastrophic situations in terms of the legend) are registrated along highways and railways, around industrial zones. Less polluted are new housing blocks and gardens; the basic pollutants being Pb, Zn, Cd, Cu, Cr, Cs. However, there is no complete coincidence of mapping units on the maps with ME concentrations in plants, soils and snow because of different ways of ME accumulation in these components.

Almost the whole territory of Moscow and Novgorod is considered to be the zone of toxic ME predominant accumulation, which is quite distinct in all the landscape components and functional zones. However, a zonal pattern may be revealed in technogenic anomalies structure. The historical centres are peculiar by their widest spectra of ME. The highest

concentration clarks (CC) in soils, as compared to literature data, are proper to Ag, Sn, Pb, Zn, Cd, W, Ib (CC 10.0 ... 3.5). On the anomaly peripheries only 2-3 ME are dominant (Pb, Zn, rarely - Cd).

Biogeochemical data demonstrated a difference in feedback effects of tree species most common in towns. Thus, popular leaves concentrate more ME than those of lime. Dandelion proves to be a leader among herbs (5 to 10 ME are concentrated), alongside with some lawn cereals. Vegetables (cabbage, carrot) are actively accumulating Pb, Zn, and Cd (6-12 clarks). These and other data indicate the formation of strong biogeochemical anomalies in towns. Therefore, sanitary control measures are urgent, unfortunately, they are far from being a routine procedure. Some practical measures are recommended to minimize the pollution in urban ecosystems.

ANALYSIS OF Pb AND Cd DISTRIBUTION IN SOIL-PLANT SYSTEM

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Specific ecological norms for pollutants should be determined accounting the mechanisms of migration and distribution of these substances between the various components of biogeocenosis. To study distribution of heavy metals (HM) in the soil-plant system we performed the experiments in different regions varied from each other by a level of anthropogenic effect: Moscow region (Prioksko-terrasny state nature reserve, Zaokskoye forestry, experimental field station belonged to the Institute of Soil Science and Photosynthesis), Czechia (meadow experimental station "Kamenicky" and the sites of degraded pine forest in Beskidy mountains), Caucasus nature reserve (Subalpine and Alpine meadows and fir forests of landscape station "Dzhugha"). The content of Pb and Cd was determined by direct atomic-absorption method with powders being atomized electrothermically (within the range 0.01-1 mg/kg for Pb and 0.005-0.01 mg/kg for Cd).

The content of Pb and Cd in the soils of Moscow region was low and amounted 6-18 mg Pb/kg of dry soil, 0.03-0.7 mg Cd/kg. The content of HM to be tested in soils of the mountains of Caucasus nature reserve was 13-36 mg Pb/kg and 0.07-0.53 mg Cd/kg; in the rock 4-35 mg Pb/kg and 0.01-0.8 mg Cd/kg (except 1 point where the content of Pb in soil reached 375 mg/kg). The content of Pb and Cd in soils of Czechia was higher: 95-550 mg/kg for Pb and 0.5-3 mg/gk for Cd. The concentrations of these pollutants decreased sharply (10-40 times) along the soil profile and they became comparable with their content in the rock. This testifies that the main mass of the heavy metals in the upper layer of the technogenic soils was formed due to anthropogenic supplies. The content of Pb and Cd in alive biomass of plants, mostly of grassy ones, changed within the range: 0.5-17.5 mg Pb/kg and 0.01-0.87 mg Cd/kg of dry plant weight. The middleweight concentration of HM in the line biomass-deadwood-litter changed in the following way: 2.1-2.6-4.0 mg/kg for Pb and 0.14-0.20-0.26 mg/kg for Cd. The accumulation of the tested HM in specific plants did not depend on their content in soil.

As seen from the calculation, the build-up factor for HM (K) (the ratio between HM concentration in plants and in soil) do not differ significantly for the plants tested at the similar concentration of HM in soil. Applying of fertilizers in agrocenosis increases K for Pb; this effect for Cd is expressed to a lesser extent. The dependence of K for tested HM on their contents in soil can be expressed by the following equation:

for Pb $\ln K = 0.02 - 0.84 \ln[Pb]$ ($r = -0.83$),

for Cd $\ln K = -1.66 - 0.78 \ln[Cd]$ ($r = 0.72$),

where [Pb] and [Cd] are the Pb and Cd concentrations in soil (mg/kg).

The analysis of these equations and the data available attests that at concentrations of HM in soil equaled to maximal permissible concentrations in Russia (20-32 mg/kg for Pb and 5 mg/kg for Cd) K are approximately 0.05-0.08 for Pb and 0.05 for Cd. This corresponds to the HM content in plants at the level of minimal values suggested as maximum permissible concentrations of Pb and Cd in plants (2-50 mg/kg for Pb and 0.2-3 mg/kg for Cd according to different literature sources).

INDICATION OF ECOSYSTEM POLLUTION ACCORDING TO THE STATE OF VEGETATION COVER

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Studying the processes of pollutant's introduction into the components of ecosystems, their migration and transformation is a complex problem. Both the knowledge of physical, chemical and biological laws, governing the trends and intensity of these processes, and the analysis of geographical situation and environmental conditions, under which the processes take place may change considerably, are important there.

Vegetation takes an active part in the interception of pollutants from air, water and soil and in further migration and transformation of these substances. Being one of the most physiognomy components or an ecosystem vegetation as a whole, as well as individual plant species, can indicate rather adequately the deterioration of ecosystems.

Approach

The investigations were carried out at the Cola peninsular of Russia. Tundra ecosystems affected by the Severonickel plant in the town of Monchegorsk were studied. The state of vegetation cover was analysed as an indicator of the state of ecosystems. The problem of criteria selection for the assessment of the state of ecosystems in the areas with different levels of technological impact is very important from the methodological point of view. The most common criteria include the florists composition of the vegetation component of ecosystems, its productivity, the volume of phytomass and its structure. Rather than absolute values of phytomass, the ratio of its fractions, such as alive/dead, green/perennial, underground/surface, is often much more important. It is precisely these indicators that can evidence the strategic changes in the ecosystems' existence under the stress conditions.

The main results

While choosing the key areas and ecosystems for further studies it is necessary to account for the influence of geographical factors. In the mountainous regions they are extension of ridges and their elevations which cause the redistribution of rainfall, from the micro-climate and the specific wind regime, etc. Depending on the degree of impact from the plant the species composition and the structure of communities change, as well as the nature of production processes. Typical representatives of tundra vegetation such as mosses and lichens, are the first to disappear. At the same time the cenotic importance of perennial low bushes and some grasses could increase. In this case the total volume of phytomass increases considerably too, but its structure undergoes serious transformations producing the changes of the processes of biological turnover and functioning of ecosystems.

SYMPOSIUM B2

INFLUENCE OF THE ENERGY RELATIONSHIPS OF TROPHIC LEVELS AND OF ELEMENTS N BIOACCUMULATION

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A concern of ecotoxicology is to predict to which trophic levels bioaccumulation of elements occurs. Transformity, a measure of the energy required to produce and maintain a component or a flow resulting from an energy transformation process, may help correlate the biogeochemistry of elements with their tendency to bioaccumulate. This notion derives from two concepts. First, common substances are more likely to be processed by the biosphere. If a substance is rare, organisms of low trophic levels may tend not to process it and it may go on to higher trophic levels (biomagnification). Its uptake by organisms of low trophic levels, however, makes it more readily available and thus less unusual to organisms of high trophic levels, that may evolve a capability of processing it. Alternatively, a rare substance may tend to be taken up directly from the physical environment by organisms of high trophic levels (bioconcentration). Second, transformity expresses energy relationships between parts of a system. Substances that require more energy to form or concentrate are also the more unusual ones. We hypothesized a correlation between the rarity, complexity and energy required for concentrating a substance, and thus its transformity, and the transformity of the trophic level to which it bioaccumulates.

We tested this hypothesis for a set of elements with published data on their biogeochemistry and bioaccumulation and on energy transfers between trophic levels in ecosystems. We calculated the transformities of the elements from the energy required by the biosphere for maintaining a difference in concentration as compared to its physical environment, and the transformities of corresponding trophic levels from the energy driving the energy flows. There was a significant rank correlation between the transformities of elements and the transformities of trophic levels to which they bioaccumulate. This relationship may be an important generalization in ecotoxicology, because it may help predict bioaccumulation tendency. However, sampling strategies used in the published studies that we consulted were not aimed at relating bioaccumulation to energy relationships. To seek confirmation of this correlation, we are carrying out a unified study to measure biogeochemistry, bioaccumulation, trophic transfers and energy sources in one single ecosystem. This involves calculating the transformity of trophic levels in the river Steina, Germany, and measuring the concentration of various elements in organisms from the trophic levels. Results of this study will be presented.

PATHWAYS OF HEAVY METALS TO SMALL LAKES IN NORWAY

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The southern part of Norway is considerably influenced by long range atmospheric transport of acidifying substances and some heavy metals from other parts of Europe. This has led to enhanced levels of the heavy metals concerned in surface soils and generally increased mobility of metals in terrestrial and aquatic systems. Under these circumstances increased input of heavy metals to lakes may occur through 1) supply through direct deposition on the lake surface or surface runoff from the catchment, 2) release from soil minerals through enhanced weathering, or 3) accumulation in the organic surface horizon of natural soils and subsequent leaching of inorganic or organically complexed forms of the metals.

In the present paper the significance of long range atmospheric transport to the heavy metal load of small lakes and the relative importance of the above processes to the supply of individual elements to the lakes are discussed on the basis of I) relations of sediment profile distributions of Pb, Hg, Cd, and Ni with other lake parameters and with atmospheric deposition data for the metals concerned, and II) correlations of total water concentrations of Zn, Pb, and Cd with other water chemistry parameters such as TOC and pH, atmospheric deposition patterns, and levels of the same elements in natural surface soil.

The experience from this work is compared with corresponding studies in other geographical regions with similar problems. Moreover the results are discussed in relation to the present state of the art with respect to the knowledge on metal cycling in surface fresh waters, i.e. regarding the importance of adsorption reactions, the relative significance of biotic and abiotic surfaces, and metal exchange at sediment-water interfaces.

TRACE METAL CYCLING IN A MEROMICTIC LAKE: THE INFLUENCE OF HYDROUS-IRON PARTICLES AND DISSOLVED ORGANIC MATTER.

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Paul Lake is a small meromictic lake located at UNDERC (University of Notre Dame Environmental Research Center) in upper Michigan. In June 1994, we investigated the chemistry of the water column in order to quantify the importance of the permanent oxic/anoxic boundary on internal trace element cycling.

CTD-O_2-pH profiles, taken from the central and deepest location, were used to determine the extend of the oxic/anoxic transition. Water samples were collected, every 0.5 m, from a small boat by peristaltic pumping. They were analyzed for major, minor and trace elements using conventional techniques as well as Capillary Electrophoresis (CIA), Voltammetry, and ICP-MS. We report here the depth profiles of T[°C], O_2, pH, Alk_T, DOC, Ca^{2+}, Mg^{2+}, Na^+, K^+, SiO_2, Cl^-, NO_3^-, $SO_4^{=}$, ΣHS^-, Fe^{2+}, Fe_p, and the trace elements Ba, Co, Sr, and Pb.

These profiles show that the oxic/anoxic transition in the water column influences drastically the mobilization of some species. For example, the concentration of K^+ increases from 20 µM to 90 µM in less than 2 meters; the concentration of Co increases from 0.25 nM to 6.5 nM just below the oxicline and follows closely the distribution of Mn^{2+}. The roles of particulate iron oxides and biogenic organic matter on the internal cycling of the major and minor elements are discussed.

MICROZOOPLANKTON GRAZING ACTIVITY INCREASES TRACE METAL RESIDENCE TIMES IN NATURAL WATERS: LABORATORY AND FIELD EVIDENCE

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What controls dissolved trace metal concentrations in pelagic surface waters? Recent measurements in the Laurentian Great Lakes of North America reveal very low levels of dissolved trace metals, approaching those found in the open ocean. Metal fluxes in this environment are determined primarily by atmospheric inputs and particle fluxes to the sediment. Current approaches for estimating trace metal concentrations and residence times are based on surface complexation modelling (SCM).

The majority of particles in the pelagic zones of large lakes are biotic - among the most numerous and productive are the picoplankton (bacteria and algae, 0.2-2 μm). The elevated surface area:volume ratio of the picoplankton results in a high potential to scavenge trace metals from the dissolved phase. The ecological fate of this plankton is predominately consumption by microzooplankton (2-200 μm; e.g. heterotrophic nanoflagellates, ciliates, rotifers). This micrograzing activity might reasonably be expected to convert trace metals from the particulate to dissolved phase, and to increase the availability of metal-complexing organic ligands in the dissolved phase. These influences would tend to increase trace metal residence times and concentrations in surface waters in a manner unaccountable by current predictive models based on SCM. Evidence for this activity is presented from a laboratory model of a simplified microbial food web and from a field study on the fate of radiolabelled picoplankton in the natural plankton community of pelagic Lake Erie, the smallest of the Laurentian Great Lakes.

Rapid regeneration of trace metals from the particulate to the dissolved phase (<0.2 μm) was observed in the laboratory with a simplified microbial food web composed of the mixotrophic chrysophycean nanoflagellate (*Ochromonas danica*) grazing on picocyanobacteria (*Synechococcus leopoliensis*) that had been previously exposed to the trace metal radionuclides ^{153}Gd(III), ^{65}Zn(II), ^{109}Cd(II) and ^{137}Cs(I). These trace metals were chosen to represent a range of surface reactivities with particles (Gd > Zn, Cd >> Cs). Grazing experiments and the appropriate non-grazing controls were carried out in batch cultures over 43-49 hours in defined, inorganic freshwater medium; metal partitioning among the consumer, prey and dissolved phases was determined by sequential filtration (3 μm, 0.2 μm) at timed intervals. The majority of trace metals consumed as radioactive prey were in fact regenerated into the dissolved phase. Regenerated Gd, Zn and Cd present in the dissolved phase were less available for resorption by plankton than were the same radionuclides added in inorganic form to fresh growth medium.

The regeneration of trace metals from prey by microzooplankton, as observed in the laboratory, was confirmed in the field. The influence of the natural Lake Erie planktonic community on the fate of trace metals was evident in the rapid remineralization of radiolabelled picoplankton added to lake water (<210 μm) containing the complete microbial community. The disappearance of radionuclides from the size fraction containing the radiolabelled picoplankton spike (0.2-3 μm) was matched by the appearance of these radionuclides in the dissolved (<0.2 μm) phase. In contrast, the amount of radionuclides desorbing from the

radiolabelled *Synechococcus* in the control treatment was markedly lower. The grazing rate of picoplanktonic chlorophyll (measured using an independent technique) matched the disappearance rate of radionuclides in the picoplankton size fraction of the natural water containing the radioactive picoplankton spike. We conclude that the natural microzooplankton (dinoflagellates, mixotrophic and heterotrophic nanoflagellates, ciliates, etc.) in the Lake Erie pelagic zone were actively grazing the radiolabelled *Synechococcus* and regenerating the radionuclides into the dissolved phase at rates comparable to their grazing of these prey.

Our results suggest thatmicrozooplankton grazing activity in natural waters will serve to increase trace metal residence times in the water column of aquatic systems.

HEAVY METAL EVOLUTION IN SEDIMENTS AND INTERSTITIAL WATERS SAMPLED IN THE RIVER SEINE DOWNSTREAM PARIS

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The important urbanised and industrialised district of Paris is one of the main "non-point sources" of heavy metals for the River Seine. In order to assess the impact of such urban runoff on the river ecosystem, sediment cores and interstitial waters, sampled with peepers, have been collected downstream Paris. Particulate and dissolved metal profiles have been built, and attempts have been made to understand the pollutant behaviour during the sediment diagenesis. One of the questions addressed during this work is the role of River Seine water-sediment interface as source or sink of trace metals: is the large amount of trace metals, released during iron and manganese oxy-hydroxide reduction, indeed released into the water column, or continuously coupled to sulphides produced by sulphate reduction ?

If *in situ* observations have been found quite informative with lake sediments, they have proven to be much more complex to interprete in large rivers running through industrialised areas and presenting large flow rate variations. This has been especially the case with River Seine which flow rate in Paris decreases in summer till ca. 100 m^3/s, whereas during autumn or winter wet periods it may overcome 1,000 m^3/s. For example, the consequence of the first flood, after the summer dry weather period, on particulate heavy metal profiles has been observed in this work. It has been described as a shift to totally different level and pattern of trace metal concentrations in less than a one month period of time. The zinc/lead ratio has been found quite indicative of suspended solid (SS) origin.

Furthermore, the ability for river sediments to move easily may distort the results obtained with peepers, for this kind of sampler is introduced into the sediment and equilibrates for an average of 15 days. Thus both sediment cores and interstitial water profiles present frequently, in Paris area, large heterogeneity (Figure 1, left) which forbids any significative assessment of concentration gradients and diffusion fluxes. Moreover, in the design of the peepers we used, two opposable sampling sides exist. The local condition, as the water flow, as well as the typical heterogeneity of the natural substratum, extend the variability between profiles obtained on both sides (Figure 1, left).

To overcome all these problems, it has been attempted to set up a laboratory pilot simulating the conditions occurring in the river Seine bottom sediments, after the input of storm overflow polluted SS. A 10 l reactor, which sides were designed as those of peepers, has been filled with an upstream Paris River Seine sediment, mixed with ca. 33% urban runoff SS and covered with bulk river water. The whole apparatus has been left, during 3 months, to evolve under a nitrogen atmosphere, while the water was constantly circulating and reoxygenated.

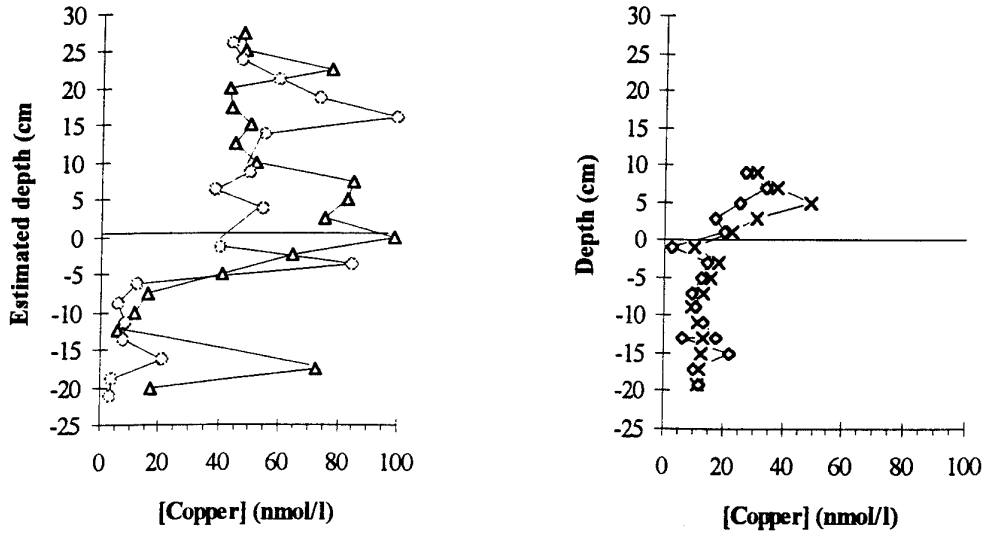

Figure 1. Dissolved copper profiles in River Seine sediment interstitial water sampled either downstream Paris in Chatou [lef: (triangles) peeper side 1 and (circles) 2] or in a laboratory reactor filled with River Seine sediments and Clichy urban runoff suspended solids [right: (diamonds) pilot side 1 and (crosses) 2)]

Figure 1 compares the ensuing dissolved copper profiles, respectively *in situ*, and in the reactor. The average interstitial water heavy metal level, obtained by the laboratory pilot, appears to be equivalent to the field observations, i.e. in the 2-20 nmol/1 range. Whereas *in situ* profiles present large heterogeneity and significant difference between both sides, pilot ones are quite homogeneous. This allows a better assessment of data precision.

Furthermore, the mild re-aeration procedure set up in the pilot has been found valuable for simulating water-sediment interface, especially the redox gradients assessed by sulphate/sulphide or nitrate/ammonium ratios. Correspondingly, the resultant experimental interface show clearly the existing heavy metal gradient between sediment and water, whereas the field measurements present much more variable concentrations at this level (Figure 1). This difference may be due to the bioturbation, more important *in situ*, and quasi non-existent in the reactor, but also to sediment transport heterogeneity.

In conclusion, we have found valuable to associate *in situ* observations to laboratory reactor ones: further experiments are in progress for attempting to determine kinetics of evolution of river sediment as well as of fluxes of trace metals from/towards the water column.

HEAVY METAL AVAILABILITY IN WETLAND SOILS

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Management of liquid and solid wastes is an on-going concern to government bodies and regulatory authorities throughout the world. These concerns, coupled with increasing public awareness, require that environmental engineers and scientists clearly understand the behaviour and fate of contaminants in wastes which are disposed of within the environment. This understanding is necessary to avoid or minimise degradation of the receiving soil and water environments. One method of treating wastewater prior to discharge involves the use of artificially constructed wetlands. As wastewater passes through the wetland system, it is exposed to a range of chemical, biological and physical conditions, which can effect contaminant solubility through adsorption, precipitation, dissolution, complexation, and degradation processes.

The main focus of this research was to indentify the important mechanisms affecting the solubility of P, Cu, Pb, Zn, Cd and Cr as they pass through a wetland. Specifically, the objectives were to (a) monitor the behaviour of P, Fe, Mn, Cu, Pb, Zn, Cd and Cr under controlled laboratory conditions similar to those occurring in natural wetlands, and (b) monitor the partitioning of metals in various geochemical fractions under varying redox and pH conditions.

Soils were initially treated with ammonium phosphate (AP) and/or heavy metals Cu, Pb, Zn, Cd, and Cr. Treated and untreated soils were waterlogged for 21 days by flooding with deionised water. Soils were then allowed to dry at room temperature over the following 21 days. Soil samples were collected on a weekly basis and analysed for pH, Eh, P, Cu, Pb, Zn, Cd, Cr, Fe and Mn. Metals were extracted using a sequential extraction technique which extracted metals from the soluble/exchangeable, carbonate, organic, sesquioxide and residual fractions. Experiments were conducted at initial soil pH values of 3, 5 and 7 to simulate the range of pH environments of the study area generated through oxidation of FeS_2 minerals.

Waterlogging for up to 21 days increased water soluble Fe, Mn and P concentrations, increased soil pH and decreased soil Eh, compared to initial values. Air drying the waterlogged soils caused the concentrations of Fe, Mn and P to decrease, and the soil pH and Eh to return to levels observed at the commencement of the waterlogging experiment. Analyses are currently underway to determine which geochemical fractions the metals are associated with.

THE EFFECT OF ROOT METABOLISM ON THE POST-DEPOSITIONAL MOBILIZATION OF MERCURY IN SALT MARSH SOILS

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Waterlogged soils are generally anoxic just below the surface. Plants growing on such soils avoid the toxic effects of anoxia by creating oxidized rhizospheres through oxygen translocation via well developed aerenchima, and exudation by roots. Therefore the creation of oxidized microenvironments into a general anaerobic soil, may result in the post-deposition mobilization of various substances, including heavy metals, in particular those like Hg which form sulfides under anoxic, sulfidic conditions, typical of salt marsh soils.

We present here the distribution of Hg in soil cores taken under *Spartina alterniflora* Loisel. marsh and compares with adjacent mud flats without plant cover. Both soils are submitted to the same pollution sources and show the same granulometry, inundation pattern and salinity regime.

Four cores were collected in each area by inserting 60 cm long plastic tubes (9 cm in diameter) into the soil. Two additional cores were taken for organic matter content and root biomass determinations. Cores were immediately taken to laboratory and sliced no later than 6 hours from sampling. In the field, plant density and height were measured for further determination of community biomass through allometric relationships with a 25 plants sub-sample dried and weighted. Also, at root depth (2-10 cm), roles were dug for the *in situ* determination of pH, platinum electrode redox potential and sulfide concentrations using portable electrodes, except for sulfides which were fixed in the field with ZnOAc 5N and determined colorimetrically.

Mercury concentrations in soil layers were determined after acid extraction, by cold vapor atomic absorption spectrophotometry. Reagent blanks and intercalibrated sediment samples provided by the Institute of Soil Fertility Research, Holland, were run simultaneously for precision determination of the methods used. All blanks gave Hg concentrations smaller than half of the lowest Hg concentrations found in samples. Intercalibration with intercalibrated reference samples gave values in the range of 10% maximum of the reported concentration.

The vertical distribution of Hg in the sediment cores (mud flat and *Spartina*) were significantly different for most of the depths sampled. At the surface of cores both sediments presented an impoverishment of Hg probably due to the intertidal conditions of the samples which may remobilize Hg to the water column. From 2 to 10 cm of depth, Hg distribution was symmetric between the two cores. Mercury concentrations in *Spartina* cores significantly decrease from 115 to 52 $\mu g.kg^{-1}$ along this depth layer, whereas in the flat cores Hg concentrations increase from 102 to 137 $\mu g.kg^{-1}$. This layer under *Spartina* presented the highest biomass of roots.

The oxygen excretion by the salt marsh roots results in a relatively oxic environment, although reducing conditions can also be developed.Typically, Eh values at this depth ranges from -110 to +110 mV, with an average of +5 mV (n=15). The excretion of oxygen also results in low concentrations of sulfides (*c.a.* 0.6 $mg.l^{-1}$).

In the mud flat cores, reducing conditions are dominant with Eh values ranging from -110 to -300 mV and an average of -204 mV (n=15). Sulfide concentration is very high reaching an average of 47 $mg\ l^{-1}$.

Below 10 cm, Hg concentrations under *Spartina* increase steadily from 95 to 162 $\mu g.kg^{-1}$, whereas in the mud flat cores Hg concentrations decrease from 137 to 61 $\mu g.kg^{-1}$.

The effects of the metabolism of the salt marsh roots clearly result in the post-depositional mobilization of Hg. The dynamic oxic-anoxic conditions at the root depth hampers the immobilization of Hg as Hg-sulfides, resulting in the migration of soluble Hg up and down the core, and a clear enrichment at deeper layers. In the mud flat cores permanent reducing conditions result in a gradual accumulation of Hg in sub-surface layers of the sediment, and a decrease in concentrations at deeper layers, since most of the accumulation is probably of highly stable Hg sulfides.

Concluding we have shown here that salt marsh plants are able to mobilize deposited Hg through the exudation of oxygen to the sediment and avoiding the development of permanent reducing conditions and the consequent immobilization of Hg as stable sulfides. Mercury remobilized through this mechanism later accumulated in deeper layers of the sediment.

VARIABILITY OF TOLERANCE TO COPPER IN FRESHWATER PERIPHYTIC COMMUNITIES

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Communities in streams are subject to chronic exposure to toxicants, which affect them functionally and structurally. If the stress is strong enough, toxicants will exert a selection pressure on the communities. As a result the least sensitive organisms will adapt better than the more sensitive ones. In comparison to previously unexposed organisms, they will become more tolerant to the toxicants. An increased tolerance to toxicants has been postulated as evidence that a community is affected by these toxicants. Therefore, tolerance measurements along pollution gradients in the field may be used to evaluate whether the presence of a toxicant has resulted in adaptation of a community and has thus had an impact.

Some streams have elevated concentrations of heavy metals, which can act as agents of selection on communities. Such communities are expected to show an increased metal tolerance and an altered community composition. In order to evaluate increases of tolerance, knowledge is required on the variability in tolerance of communities not exposed to the metals. In this study we investigated the tolerance to copper of freshwater periphytic communities of rock surfaces, from various habitats.

Periphytic communities from the river Töss, Switzerland, were assayed in terms of the sensitivity of their photosynthetic activity to copper exposure, as determined by the ^{14}C-incorporation method. The variability of tolerance to copper was determined by comparison of the copper concentrations inhibiting 20% of the photosynthetic rate (IC_{20}) after 1 h of exposure. In order to reduce another source of variability, that is, that originating from the differences in water chemistry at the various sites, the communities were assayed in a synthetic medium that allows calculating the copper speciation. The communities investigated so far differed little in their sensitivity to copper. The 1-h IC_{20} ranged from about 0.1 to 1 μM copper. Considering also that these communities appeared to be different in their taxonomic composition, it seems that different periphytic communities not previously exposed to the metals are similar in their tolerance to copper. More communities are currently being tested.

The tolerance values found in this study will be compared to values for communities from streams having elevated copper concentrations. Given the low variability of copper tolerance in non-exposed communities, it is concluded that measurements along copper gradients in streams may be useful in the detection of negative impacts on communities.

METAL ACCUMULATION BY SEDIMENT ORGANISMS IN RELATION TO METAL SPECIATION IN THE SEDIMENTS

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Introduction

In studies of accumulation and biological effects of metals on aquatic organisms, free-swimming organisms are usually used. However, a great amount of metals in natural aquatic systems binds to particles and sooner or later ends up in sediments. Metal-uptake by sediment-dwelling animals (zoobenthos) which hide in and eat from the sediment, is presumably favoured by the combination of high metal concentrations in their diet as well as in their physical environment, and higher ingestion rates necessitated by the low food quality of detrital organic matter. Furthermore, for several years it has been stressed that measurements of total concentrations of metals in sediments reveal little about the availability of these metals. All the same, total concentrations are often the parameters considered in risk assessments. Therefore it is important to evaluate test-systems for metal contaminated sediments, in which effects on sediment-dwelling organisms are studied in combination with speciation of the sediment associated metals.

Objectives

The objectives of the present study are to a) identify bioavailable fractions of metals in sediments and b) investigate how changes in environmental conditions, such as pH, redox potential (Eh) and trophic status, affect the availability (and speciation) of the sediment associated metals. At the moment of writing, most evaluated results are on mercury (Hg), but results from ongoing similar studies with cadmium (Cd), copper (Cu) and lead (Pb) will be presented at the conference.

Methods

To evaluate which factors that determine metal-accumulation in sediment-feeding animals (detritivores), laboratory experiments were carried out in which the accumulation of metals by chironomid larvae (*Chironomus riparius*) from different types of natural sediments was studied. The accumulation was related to the speciation of the metals in the sediments and also to other sediment parameters such as pH and Eh. Centrifugation of the sediments was used to separate interstitial water from particulate sediment fractions. Chemical extraction procedures were then applied for the speciation of metals associated with the particles. The following extraction chemicals were used: distilled water (water soluble fraction), 1 M NH_4Ac ('exchangeable fraction'), 0.1M $(NH_3OH)Cl$ (reducible fraction), 1 M HCl (readily extractable, reducible, non-detrital fractions), and 1 M NaOH (metals bound mainly to humic material). The sediments were also analysed for methyl-Hg. Laboratory results were compared with field-data for chironomids and surface sediments sampled in different lakes.

Results

Bioconcentration (BCF) of Hg by the chironomids was always higher from meso- and dystrophic sediments than from eutrophic sediments and sediments contaminated with Hg-rich fibre from paper and pulp industry. Methyl-Hg in sediment was not the major contributor to the Hg-accumulation by chironomids. Among the studied association forms, the relative amount of sediment-Hg extractable with 0.1 M NaOH, showed the strongest positive correlation with the accumulation in chironomids. Hg in interstitial water and Hg bound to reducible compounds in the sediments did not significantly affect the Hg-accumulation. Lowering of pH as well as an increasing the Eh in the sediments caused a redistribution of sediment-Hg, resulting in more Hg by the chironomids increased. The laboratory results were confirmed with the field data. Preliminary results for the other metals also show that the NaOH-extractable form is the most bioavailable.

CONCENTRATIONS OF COPPER IN SEDIMENTS AND FISHES FROM KAOHSIUNG RIVER AND HARBOR AREA, TAIWAN

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Over a number of years the Kaohsiung river has received waste discharges containing metals such as copper (Cu). Since 1988 Chi-Ping-Chau Barrier has built as the first sewage barrier gate to cut and redirect the upstream waters. Downstream the Barrier, along the River several sewage barriers have also set to cut off various untreated waste waters. At the present time in rain season of spring and summer, the section downstream the Chi-Ping-Chau Barrier is subject to untreated storm water discharges.

From June 1993 to September 1994 Cu levels were examined seasonally in sediments, collected from 13 stations on the stretch of the River that runs through the city of Kaohsiung. The sediment samples were dried at 105°C, after grinding, the fraction smaller than 63 μm was used for analysis. A total of 16 local edible fishes were also collected from the same area. According to the size of fish, fish were either dissected into several parts, namely, gill, liver, intestine/viscera and body carcass, or whole fish digested. Fish samples were dried at 70°C to constant weight. Sediment and fish samples were wet-digested either with combination of HNO_3 and HCl, or HNO_3. Then, the samples were measured by Atomic Absorption Spectrophotometry (Hitachi, Z-8200). Several certificated standard materials (PACS-1, DOLT-2, and DOLM-2 from National Research Council of Canada; NIES No.2 from Japan Environment Agency; MA-A-2 and SL-1 from International Atomic Energy Agency, Monaco) were used and satisfied precision (generally , ±5%) were obtained.

Upstream and downstream of the River near background levels of Cu (40-70 mg/Kg dry wt.) were found in sediments, but concentration exceeded 1000 mg/Kg at the vicinity of Chi-Ping-Chau Barrier. Seasonal Cu variation were also revealed in the sediments from three stations, the most upstream station, the outlet of the harbor and the Chi-Ping-Chau Barrier ($P<0.001$). We found that there is a metal sink in the middle section of the River, downstream the Chi-Ping-Chau Barrier, and the harbor, where average Cu concentrations were between 267 to 745 mg/kg dry wt. These results were generally 2 to 3 fold higher than Chen's (1977) investigation, except the highest Cu value found at the Chi-Ping-Chau Barrier which was 10 times higher.

Among the 16 fish species analyzed, there was a significant difference between different tissue. The fish carcasses were always showed the lowest Cu concentrations, ranging 1.7 to 3.2 mg/kg dry wt. Except the *Scatophgus argus* and *Siganus fuscescens* had the highest Cu content in their viscera, fish liver had the highest copper concentrations (8.8 to 91.4 mg/kg dry wt.).

Species differences were found in relation to habitat and feeding habits of the fish, generally demersal and sediment-contacted fishes had higher Cu burden. Fishes lived in the metal sink tended to accumulate more Cu in their body.

Two local dominant fish species, *Liza macrolepis* and *Nemalatous comes*, were selected for investigating the relationship between the Cu body burden and fish size. These two

species are both benthic algae, detritus, and sediment feeders. The importance of sediment-route exposure is discussed.

FUNDAMENTAL LINKS BETWEEN MERCURY CHEMICAL SPECIATION IN THE AQUATIC ENVIRONMENT AND BIOACCUMULATION MECHANISMS

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Mercury occurs in various physical and chemical forms in the environment - elemental Hg, inorganic and organic compounds -, resulting from complex abiotic and biotic transformations. These different chemical forms are characterized by specific ecotoxicological properties, regulating the metal partitioning and bioavailability within the biotopes, bioaccumulation and trophic transfer capacities, combined with toxicological effects.

Several examples of results from experimental studies, based on indoor microcosms with different biological models - rooted macrophytes, burrowing insect larvae, molluscs, crustaceans, ... - clearly show the marked effects of the abiotic factors - temperature, pH, pCl, ... - upon mercury bioaccumulation, from the water column or sediment as initial contamination sources.

The physical-chemical characteristics of the medium are able to act simultaneouly on the structural and functional properties of the living organisms, notably at the biological barrier level (interfaces between organisms and the surrounding medium), and on the metal chemical speciation within the biotopes. Inorganic mercury (HgII) and methylmercury (MeHg) are present in the dissolved phase of the aquatic environment as more or less diversified set of chemical speices, resulting from a large number of chemical equilibria with coordinating ligands in solution, major complexes being controled by pH and pCl: $HgCl^2$, $HgCl^+$, HgOHCl, $HgOH^+$, $Hg(OH)_2$, $HgCl_3^-$, $HgCl_4^-$, ... for HgII; CH_3HgCl, CH_3HgOH, CH_3Hg^+ for MeHg.

Our knowledge of these speciation processes is essentially based on thermodynamic equilibrium calculations (MINEQL computer programme for example). Al though chemical modeling is well designed to deal with homogeneous solution chemistry, it cannot easily accomodate heterogeneous processes, especially if dissolved organic ligands (humic substances for example) or/and particulate ligands (suspended matter, living organisms) are present. Recently, high resolution ^{199}Hg NMR, a non-invasive technique, has made it possible to monitor HgII chemical speciation and to follow metal binding properties towards cell membrane ligands. The presence of Hg chemical species and their relative abundance could be strongly modified for slight variations of the physico-chemical characteristics of the medium, variations which are frequently encountered in natural conditions: lake acidification, pCl gradients in estuaries, ... These chemical species have different properties, notably in relation to their electrical charges (neutral, anionic and cationic species) and their solubility. For example, octanol-water partition coefficients for MeHg reveal strong differences between CH_3HgCl and CH_3HgOH species, KOW measurements being close to 1.7 and 0.07 respectively.

Results from experimental studies based on membrane models (lipidic dispersions, liposomes, micelles, BLM) and biophysical techniques (fluorescence polarisation, NMR, ...) show important differences between HgII and MeHg chemical species relating to the metal accessibility to the hydrophobic core of phospholipidic bilayers, transmembrane fluxes, binding to membrane ligands (phospholipid polar heads) and structural and functional membrane perturbations (fluidity of the phospholipidic bilayer).

In conclusion, dissolved chemical species of inorganic mercury and methylmercury are able to play a fundamental role in the ecotoxicological properties of the metal, in relation to natural and anthropogenic variations in the physico-chemical characteristics of the aquatic environment,

especially at the interfaces between the surrounding medium and living organisms. Research in this field must be based on a multidisciplinary approach, in order to develop more specific and convenient speciation methods and to analyse the mechanisms involved, from the molecular and cellular basis to more complex biological models.

CADMIUM ACCUMULATION IN WATER FERN (*Salvania rotundifolia* Willd.) PLANTS

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The aquatic vascular Water Fern plant (*Salvania rotundifolia* Willd.) was investigated as a potential biological filter for removal of Cd from wastewater. Water fern plants were grown in and harvested weekly from 0.10 *M* Hoagland nutrient solutions containing 0.01 to 0.13 μg Cd mL^{-1} or 0.50 *M* Hoagland nutrient solutions containing 0.02 to 9.14 μg Cd mL^{-1}. Dry weights of plants significantly increased when exposed to 0.01 to 1.03 μg Cd mL^{-1} in 0.10 *M* Hoagland solution during first week then decreased thereafter. A similar trend was observed in dry weights when exposed to 0.02 to 9.14 μg Cd ml^{-1} in 0.50 *M* Hoagland solution. Tissue Cd concentrations in plants grown in 0.10 *M* Hoagland solution increased during the first two weeks followed by decreases in week 3 through 5. However, tissue Cd increased through week 3 or 4 followed by decrease at week 5 in plants grown in 0.50 *M* Hoagland solutions. Cadmium exposure to plants grown in 0.10 *M* Hoagland solution did not influence the tissue P concentrations in plants. Tissue P in 0.50 *M* Hoagland solution grown plants decreased after 4 weeks. Irrespective of nutrient solutions and Cd levels frond production significantly increased up to one week and decreased thereafter. These results suggest that Water Fern would be useful for absorbing Cd from nutrient-rich water when the solution concentration was in the range of as low as 0.01 and as high as 9.14 μg Cd mL^{-1}. However, the harvest regime would have to be every one or two weeks to sustain plant vigor, and realize maximum uptake of Cd from solution.

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TRACE METALS AND HISTORY: THE SEDIMENTS OF BAYOU SAINT JOHN, NEW ORLEANS, LA, USA

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Bayou Saint John is a natural channel of water that opens into Lake Pontchartrain. It is an important historic site of New Orleans. During W.W.II, the Landing Craft Vehicle, Personnel (LCVP) boats were tested on Bayou Saint John. The LCVP boats made the D-Day (June 6, 1944) landing possible on the beaches of the Normandy coast of France. In pre-Columbian America, Bayou Saint John was used by the Native Americans as a route from inland lakes to the Mississippi River. French settlers arrived in 1718 and formed a community that predates the French Quarter of New Orleans. Dwellings were built on high ground and constructed on piles at the edge of the bayou. The over water dwellings were removed beginning in the 1920's although a few remained until the 1940's. An American Can Company factory was located at the upper end of the bayou and operated from 1906-1986. There are several gas stations and automobile repair shops along the bayou. Roads run the length of the Bayou and eleven bridges cross the bayou that range in size from two-lane to multi-lane freeway structures. In addition, a double track railway bridge crosses the bayou. Although swimming is not permitted, Bayou Saint John is currently a site for recreation including hiking, jogging, canoeing, and fishing. The purpose of this study is to examine the quantities and distribution of metals in the sediments of Bayou Saint John.

Samples were collected with a Wildco Hand Core Sediment Sampler. In total, about 140 samples from 12 bridges and about 200 samples from 28 sites between bridges were collected. The samples were oven-dried, extracted for two hours with lN HNO_3, and filtered. The extractant was analyzed for 9 metals (Pb, Zn, Cd, As, Mn, Se, Ni, Fe, and Cu) using a Spectro Inductively Coupled Plasma Spectrometer.

The results show that sediments with the highest levels of the metals Pb and Zn are associated with places having the largest volumes of automobile traffic. Additional evaluation is being conducted to examine the flows of the other metals from their sources to water, sediments, aquatic organisms, wildlife, and people. The major goal is a comprehensive understanding of potential routes of metal exposure within an urban bayou.

This study has been funded by grant #U50/ATU398948/02 from the US Agency for Toxic Substances and Disease Registry.

DES OLIGO-METAUX (TRACE METALS) ET L'HISTOIRE : LES SEDIMENTS DU BAYOU ST. -JEAN, LA NOUVELLE ORLEANS, LOUISIANE, USA

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Le bayou St.-Jean, un site d'une grande importance historique, est un canal naturel d'eau qui débouche dans le Lac Pontchartrain. Pendant la Deuxième Guerre Mondiale, les bateaux Landing Craft Vehicle (LCVP) (bateaux d'atterrissage) furent mis à l'épreuve dans le bayou. Ces bateaux assurèrent la réussite de l'invasion de la Normandie du 6 juin 1944 sur les plages de la côte normande. Dans l'Amérique de la période pré-colombienne, le bayou St.-Jean permettait aux Indigènes d'avoir accès au Mississippi des lacs intérieurs. Les colons français qui y arrivèrent en 1718 fondèrent une communauté aux bords du bayou qui vient avant l'établissement du Vieux Carré à la Nouvelle Orléans. Des logis reposaient sur un terrain solide et d'autres furent bâtis sur pilotis aux bords du bayou. Des logis bâtis directement sur de l'eau furent démolis au cours des années 1920, quoiqu'il en soit resté quelques-uns jusqu'aux années 1940. Une usine de la société American Can Company fut située en amont du bayou et fonctionnait de 1906 jusqu'à 1986. Il y avait plusieurs stations-service et des garages aux bords du bayou. Des routes bordent le bayou et il y a en tout onze ponts qui le traversent, d'une importance variée de deux voies de circulation jusqu'à une structure d'autoroute multi-voie. En plus, il y a un pont ferroviaire de voie double qui traverse le bayou. Bien que la natation soit interdite, le bayou St.-Jean est un terrain de récréation comprenant des activités telles que le jogging, le canotage, et la pêche à la ligne. Le dessein de la présente étude est d'examiner les quantités et la distribution des métaux dans les sédiments du bayou St.-Jean.

Des prélèvements des sédiments ont été pris avec un Wildco Hand Core Sediment Sampler (appareil adapté à la prise des prélèvements). A peu près 140 prélèvements des ponts et approximativement 200 prélèvements de 28 sites de recherche entre des ponts ont été amassés. Les prélèvements se sont désséchés au four, soumis à l'extraction avec 1N HNO_3, et filtrés. L'extractant a été analysé pour 9 métaux (Pb, Zn, Cd, As, Mn, Se, Ni, Fe et Cu), l'analyse étant faite avec un Spectronomètre « Spectro Inductively Coupled Plasma ».

Les résultats font preuve de la plus forte présence des métaux Pb et Zn aux sites où la circulation des automobiles est la plus importante. Une évaluation supplémentaire actuelle se fait afin d'examiner le passage d'autres métaux en question de leur matière d'origine aux eaux, aux sédiments, aux organismes, au gibier, et éventuellement aux être humains. Le but principal de l'étude est une compréhension globale des routes possibles d'exposition métallique dans un bayou urbain.

Cette étude est subventionnée par la subvention No. #U50/ATU398948/02 de l'Agence U.S. pour les Substances Toxiques.

IMPACTS OF HEAVY METALS ON AN AQUATIC ECOSYSTEM IN THE VICINITY OF A LEAD/ZINC SMELTER

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Previous studies have indicated that there has been considerable contamination of the terrestrial and aquatic ecosystems within the vicinity of a lead zinc smelter and industrial complex at Avonmouth, Bristol, UK. This area is drained by numerous streams which flow directly into the Severn Estuary.

Surface water can be considered to be the most obvious medium in which to assess, monitor, and control water pollution, because the results of analysis are directly related to the potential effects in living organisms. Water samples from the various streams were analysed by ICP-AES giving a range of metal concentrations as follows; Cd, 0.005-0.250; Pb, 0.05-2.83; Cu, 0.83-0.417; Zn, 0.05-33.64 µg/g showing high levels of contamination.

A qualitative survey of the aquatic flora of this region revealed a marked difference between species diversity amongst the slow flowing streams of the area. Members of the aquatic plant community are affected to varying degrees by heavy metal pollution and this is reflected in the appearance of species in order of tolerance to the toxin. *Lemna minor*, the common duckweed, was present at varying densities in these streams. Duckweed growing in streams close to the smelter had lower chlorophyll concentrations, small fronds and low population density. In comparison the duckweed found in streams much further away had higher chlorophyll concentrations, larger and healthier fronds were observed to have a swollen appearance. Metal concentrations in two other species of aquatic plant were also observed to be higher in the streams closer to the smelter than to those further from the site.

ICP-AES analysis of the samples of *Lemna* for selected elements had the following range of metal concentrations; Cd, 3-45; Pb, 50-400; Cu, 16-57; Zn, 200-7000 µg/g/

The paucity of duckweed and other vascular plants were attributed to contamination of the water by heavy metals. Substantial evidence has shown that *Lemna* sp. is a strong candidate for air and water quality assessment. A number of methods have been developed to quantify responses of duckweed in such tests. These rely on such things as frond multiplication, frond colour, dry and fresh weight changes and chlorophyll content. A scale of the degree of damage to fronds can be applied to living samples in the filed on the basis of their colour. Thus allowing a precusory assessment of water quality. In this study samples of live *Lemna* from several locations were experimentally exposed to varying concentrations of zinc and cadmium. As metal concentrations increased a decrease in frond area, frond multiplication, chlorophyll content, fresh and dry weights were observed.

Transmission electron microscopy (TEM) and X-ray microprobe analysis conducted upon samples of *Lemna* exposed to varying concentrations of cadmium and zinc revealed

damage to subcellular organelles, principally the chloroplasts, together with areas of metal deposition.

Both field and laboratory observations have shown that the condition and appearance of *Lemna* correlates with exposure to heavy metals. The ease of culture and small size of *Lemna* sp. coupled with the extent and location of cellular damage has been used as an indicator of aquatic pollution. Further research should focus on the accumulation of other elements within cells in response and tolerance to exposure of high metal levels.

THE DYNAMICS AND FLUXES OF TRACE METALS IN AN ACIDIC STREAM AND LAKE

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After many years of research stimulated by the problems of acid rain, understanding of the mechanisms within soils controlling trace metal concentrations in streams is still poor. There are quite detailed descriptions of the behaviour of trace metal in lakes, but again a general coherent framework is lacking. A feature which has clearly emerged in circumneutral waters, however, is the importance of phytoplankton in controlling the partitioning of metals between solution and particles and determining their transport to the sediment. There are relatively few studies of trace metals in acid lakes and, partly because of the usually impoverished biota, none have investigated the role of phytoplankton.

A large scale lake manipulation study was conducted to investigate the effect of adding phosphate to an oligotrophic acid lake. It provided a unique opportunity to examine directly how phytoplankton can affect trace metal concentrations and fluxes in acid waters. Concentrations of copper, zinc and aluminium were measured in the dominant inflow and the lake water for at least a year before and after the lake's primary productivity was stimulated by adding phosphate fertilizer. Good records of flow allowed measured dissolved metal concentrations in the inflow to be used to predict dissolved concentrations in the lake. Prior to fertilization all three metals behaved conservatively, with their lake concentrations being successfully predicted. After fertilization measured dissolved concentrations were lower than predicted, especially in the summer months, indicating removal to the particulate phase. The effect was most pronounced for copper, although this was probably due to low dissolved copper concentrations (~1 $\mu g\ l^{-1}$) being sensitive to any transfer to a solid phase.

While removal of aluminium could be due to a chemical mechanism associated with the slightly higher pH, the removal of copper and zinc is almost certainly attributable to uptake by phytoplankton.

EXPERIMENTAL STUDY OF THE COMBINED EFFECTS OF pH AND SALINITY ON INORGANIC MERCURY BIOACCUMULATION AND TOXICITY TO THE TURKISH CRAYFISH (Astacus leptodactylus)

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From an ecotoxicological point of view, the assessment of metal toxicity in aquatic systems is very complex, as contamination of the living organisms results from the actions and interactions between the exposure modalities and the abiotic and biotic factors. Thus, every change in the physicochemical characteristics of the medium, wether natural or anthropogenic in origin, is able to modify the chemical fate and bioavailability of the metals, via the chemical speciation reactions, and to act jointly on the organisms, inducing adaptative responses in the physiological functions, especially at the biological barrier level.

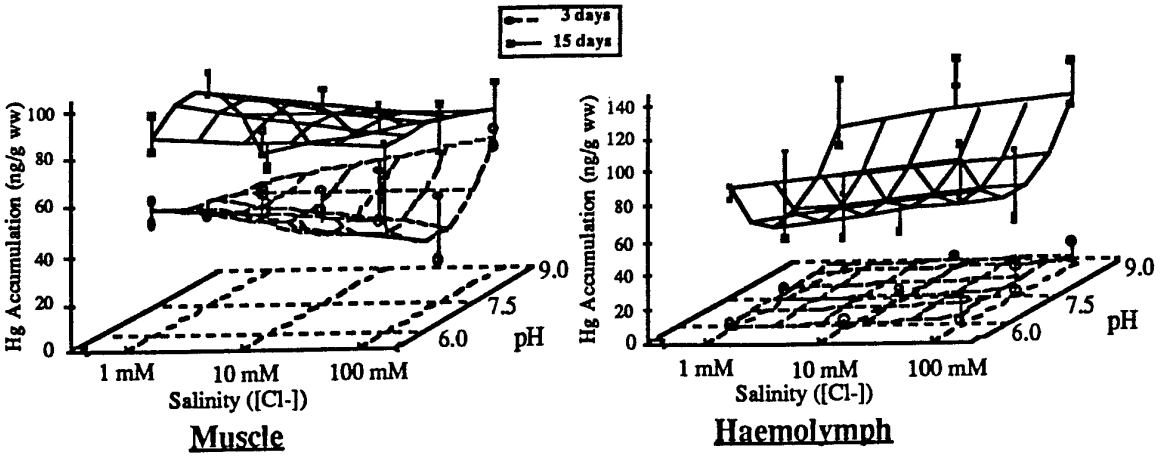
The object of the present study was to analyze the combined effects of pH and salinity on the bioaccumulation and toxicity of inorganic mercury to the turkisch crayfish: *Astacus leptodactylus*.

The experimental protocol, based on a complete factorial design, took into account 9 experimental conditions, resulting from the combination of 3 levels for pH (6, 7.5 and 9) and for salinity (1, 10 and 100 mM Cl^-). Two exposure durations were selected (3 and 15 days), with 2 replicates per condition.

Homogeneous batches of crayfish (males, intermoult stage- 30±6 g, ww) were progressively acclimatized to the 9 conditions selected, over a period of 3 weeks. Organisms were then individually introduced in the experimental units (Eus - 3 l of water); salinity levels were obtained by adding adapted volumes of concentrated solution of NaCl and pH was regulated by automized systems (controlled additions of H_2SO_4 or NaOH from diluted solutions - regulation at ± 0.3 pH unit). Eus were placed in large thermoregulated tanks (15 ± 0.2 °C) and continuously aerated, with a 12:12h photoperiod. The nominal Hg concentration was identical for all the conditions: 1 $\mu gHg.L^{-1}$. In order to avoid excessive fluctuation in the physicochemical characteristics of the medium and to minimize the decrease of the metal concentrations in the water column after the initial additions (Hg volatilization, adsorption on the walls, bioaccumulation, etc.), the medium of each EU was entirely renewed every 24h. Before each renewal, water samples were collected in order to measure Hg concentrations at the end of the cycles; data obtained enabled us to quantify the exposure conditions of each organism and to take into account, via the determination of a contamination pressure factor, potential differences between the experimental conditions. Haemolymph samples were collected at time zero and after 3 and 15 days, in order to measure the osmotic pressure (osmometry) and the principal ions (Ca^{++}, Na^+ and K^+ with flame spectrophotometry - Cl^- with coulometry). After 3 and 15 days, each crayfish was weighed and stored at -20°C. Total Hg bioaccumulation was measured at the whole organism and organ levels; gills, abdominal muscle, shell samples, digestive tract, haemolymph, hepatopancreas. Mercury determination was based on cold vapor atomic absorption spectrometry (Varian AA475), after a sample digestion step (pure HNO_3, 90°C, 3h in a pressurized medium).

The results show an important Hg accumulation after 15 days' exposure, with the organs in direct contact with the surrounding medium - gilles and shell - having the highest concentrations (2300 and 600 $ng.g^{-1}$ respectively). Comparative analysis of the bioaccumulation tendencies clearly reveals 3 groups of organs and tissues: at the gills, shell and hepatopancreas levels, the increase of

Hg concentrations according to exposure duration is close to linearity; the muscle and digestive tract follow a plateau tendency, after 3 days' exposure; lastly, the haemolymph is characterized by a biphasic acceleration. The effects of the 9 pH and salinity conditions on Hg bioaccumulation are complex and variable from one organ to another. For example, two compartments show symmertical tendencies towards their accumulation capacities after 15 days (figure below): metal concentrations in the abdominal muscle are maximal when pH is close to neutrality, whatever the salinity level, even though they are minimal in the haemolymph. This opposition may seem surprising, but we should bear in mind that these two compartments have different physiological and toxicological functions: Hg transport and exchange for haemolymph, storage for the muscle.



The relative abundance of inorganic Hg chemical species in the external medium, for the 9 conditions studied, was estimated from thermodynamic calculations (MINEQL programme). Correlations were studied between the chemical species concentrations in the water ($HgCl_2$, HgOHCl, $HgCl_3^-$, $HgCl_4^-$, $Hg(OH)_2$) and those of total Hg accumulated in the different organs and tissues. For example, a positive correlation was found between the neutral species $HgCl_2$ and the amounts of metal in the gills.

Perturbations of the haemolymph ionic parameters in response to changes in the chemical characteristics of the medium and to the mercury exposure were minimal, as the contamination pressure selected was probably too weak to induce structural and functional effects able to produce a significant perturbation of the crayfish regulatory capacities.

Complementary studies are currently under way to investigate the role played by the gill barrier towards the binding and absorption of inorganic Hg and its principal chemical species. A second biological model is also used, the green crab (*Carcinus maenas*), in order, in particular, to consider a wider range of salinity (100 to 500 mM).

EFFECT OF NOGENT NUCLEAR POWER PLANT ON RIVER SEINE SUSPENDED SOLIDS: METAL CONCENTRATION AND GEOCHEMICAL DISTRIBUTION

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Specific aims

This study was completed in order to assess the effect of Nogent nuclear plant on River Seine suspended solids (SS) ca. 150 km upstream Paris. The cooling water requirements are ca. 4 m^3/s for 2 units. The blowdown of the cooling tower is approximately 2.5 m^3/s. As an antiscaling treatment, sulphuric acid is added to water. Metals, originating from the copper and zinc alloy tubes, are carried out and disposed off downstream. This creates a potential concern of metal presence in the aqueous environment: thus, particulate metal mobility and bioavailability was firstly assessed using geochemical speciation.

Experimentals

Samples were collected from the River Seine, both upstream and downstream the plant, as well as in its outlet canal. Two types of sampler were used during a week campaign performed on November 1993:

- one continuous centrifugal collector during 3-4 h periods (9000 rpm) and
- two 70 l suspended solid traps, placed on site for 24 h periods.

SS samples were analysed for major levels of iron and manganese, as well as for traces of copper and zinc. Metal concentrations were determined by flame atomic absorption spectrophotometer. Total particulate metal concentrations were performed after strong acid mineralisation, using a mixture of concentrated fluorhydric and perchloric acids.

Five fractions for the particulate metals were identified, using Tessier (1979) speciation scheme: exchangeable, acid-soluble, reducible, oxidisable and residual.

Results and discussion

Centrifuge collected samples were found richer in organic matter than the trap-collected ones; this was evidenced by differences in COP (31 *vs.* 35 g/kg d.w.) as well as in weight loss at 550°C (80 *vs.* 130 g/kg d.w.). Correspondingly, the oxidisable fraction (representative of the SS organic phase) of all centrifuge-collected samples exhibits significantly higher copper concentration than for the trap-collected ones (4 *vs.* 18 mg/kg d.w.). This larger oxidisable fraction concentration was also observed for zinc, although less significantly than for copper. Copper has been described as presenting a typical affinity for organic matter (Hart, 1982; Pardo, 1990). Nevertheless the copper concentration difference

METHODES CHIMIQUES ET BIOLOGIQUES APPLIQUEES A L'ETUDE ECOTOXICOLOGIQUE DE COURS D'EAU DE LA REGION NORD/PAS-DE-CALAIS (CANAL A GRAND-GABARIT, RIVIERES LYS ET SOUCHEZ, FLEUVE CÔTIER AA, RIGOLES DU NORD ET DU PAS-DE-CALAIS)

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Depuis plusieurs années, le Service d'Hydrobiologie et d'Ecotoxicologie effectue des recherches sur la qualité de certains cours d'eau de la Région Nord/Pas-de-Calais.

C'est ainsi qu'ont été étudiés en des points choisis en fonction de critères bien définis le Canal à grand-gabarit, le fleuve côtier Aa, les rivières Lys et Souchez et les Rigoles du Nord et du Pas-de-Calais ; la très forte pollution de ces dernières menaçant la qualité de la nappe souterraine.

Les travaux portant à la fois sur les qualités physico-chimiques et chimiques du milieu (eau et sédiments) ainsi que sur la qualité biologique ont été effectués avec des outils variés : dosage de métaux lourds (Al, Cd, Cr, Cu, Fe, Pb et Zn), d'hydrocarbure, de PCB et de pesticides, recherche de corrélations métalliques, analyses en composantes principales, caractérisation d'indices biologiques de qualité, test « MICROTOX » etc.

Des classements intersites ont ainsi pu être réalisés en fonction des paramètres retenus et des normes en vigueur. La comparaison des résultats obtenus avec les différentes méthodes utilisées a aidé à redéfinir l'adéquation de ces dernières aux problèmes spécifiques rencontrés sur le terrain (fonds, débits, systèmes atypiques...).

Par ailleurs, l'examen du compartiment biotique a aussi permis d'analyser les transferts de micropolluants du compartiment abiotique aux organismes vivants (végétaux, macrobenthos, poissons), de repérer des indicateurs de pollution, d'apprécier l'impact des qualités physico-chimiques et chimiques du milieu sur la biodiversité et les chaînes alimentaires aquatiques régionales.

Toutes ces études ont été réalisées avec le soutien financier de l'Agence de l'Eau Artois Picardie, du Ministère de l'Environnement via la DIREN de la région Nord/Pas-de-Calais, la Société des Eaux du Nord, du Conseil Régional.

measured in the oxidisable fractions was not found proportional to the difference in COP or VSS. This indicates that the reagents used in the geochemical speciation are not entirely specific.

Particulate copper and zinc present in the plant outlet were found very variable within the week sampling period, corresponding to a specially high release of particulate metal, respectively ranging from 2,100 to 4,100 and from 830 to 1,400 mg/kg d.w. They were found to be much more labile than the samples collected in the river itself, the copper acid-soluble fraction containing 29-35% instead of 2-6% of all particulate copper. This indicates the presence of carbonate particles in this plant discharge water, which salinity, evaluated by conductivity, is much higher than River Seine one (respectively 600 *vs.* 400 µS/cm): indeed, such discharged copper presents much higher mobility.

The effect of these high copper and zinc particulate concentrations discharged by the plant is apparently small for the copper and negligible for the zinc, when assessed 1.5 km downstream the plant. Total copper concentrations increases from 20 to 44 mg/kg d.w. while zinc remains around 100 mg/kg d.w. During this wet season campaign, dilution effects have to be taken into consideration since the Seine flow of 100 m^3/s largely overcome the industrial discharge flow of 2 m^3/s.

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CARACTERISATION D'OUTILS BIOLOGIQUES (ESPECES INDICATRICES, BIOINDICATEURS DE POLLUTION, MARQUEURS BIOCHIMIQUES) ADAPTES A L'ETUDE DES EFFETS DES MICROPOLLUANTS DANS LES ESTUAIRES ET PORTS DE LA REGION NORD/PAS-DE-CALAIS

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Des études écotoxicologiques de terrain ont été menées dans les estuaires (Canche et Aa) et ports (Boulogne ^{s}/mer et Avant Port Ouest de Dunkerque) de la région Nord/Pas-de-Calais. L'impact de la qualité du milieu (eau et sédiments) sur les communautés benthiques a été recherché avec des méthodes voisines de celles utilisées par ailleurs pour l'étude de certains cours d'eau (voir Grumiaux *et al.*, ce colloque) mais adaptées au milieu marin. C'est ainsi que des tranferts de micropolluants, de métaux lourds en particulier (par exemple : Al, Cd, Cr, Cu, Fe, Pb et Zn) des sédiments aux macroinvertébrés benthiques ont été examinés et que les effets de la granulométrie des sédiments et de la charge de ces derniers en matière organique sur la répartition des espèces et sur la biodiversité ont été mis en évidence.

Afin de réaliser une analyse beaucoup plus fine des effets biologiques potentiels de la pollution des systèmes étudiés et des mécanismes de défense développés par la macrofaune associée aux sédiments, nous avons choisi comme modèle une espèce très représentative du milieu estuarien et excellent bioindicateur ; l'annélide polychère *Nereis* (*Hediste*) *diversicolor* O.F. Müller. Nous ne rappellerons ici que quelques résultats majeurs obtenus par notre Equipe.

Au laboratoire, les effets pathologiques de métaux lourds tels que Cd, Cr, IIg et Pb ont été observés en microscopie photonique et/ou électronique. La cinétique du Cd à l'intérieur du ver a pu être étudiée grâce à l'emploi du Cd^{109}.

Par ailleurs, il a été démontré par voie biochimique qu'après son entrée dans l'animal, un métal lourd tel que le Cd est complexé à deux métalloprotéines (MP) qui sont, en fait, des ferroprotéines : l'hémoglobine (MPI) et une hémérythrine (MPII). Cette dernière molécule inconnue jusqu'alors chez la néréis mais connue chez d'autres vers, les sipunculiens, comme étant un pigment respiratoire, et retrouvée depuis chez d'autres annélides, a été isolée, caractérisée, séquencée. Des anticorps poly- et monoclonaux dirigés contre la PMII ainsi qu'une sonde oligonucléotidique ont été obtenus. Ces différents outils ont permis par la méthode immunocytochimique couplée ou non à l'hybridation *in situ* de détecter les mécanismes de détoxication mis en jeu à l'échelle cellulaire ainsi qu'une réponse génomique entraînant la synthèse de la MPII en cas de contamination métallique. Il est à noter que la MPII a aussi un pouvoir bastériostatique en intervenant par un processus de ferroprivation.

Des travaux ultérieurs ont montré qu'en cas d'agression la néréis peut aussi synthétiser d'autres protéines : protéines dites de stress, système « Multi Drug Resistance » (MDR) dont la spécificité de réponse est actuellement en cours d'étude.

Enfin, la caractérisation biochimique récente des cholinestérases de la néréis permet aussi d'envisager d'utiliser ces dernières comme test de toxicité envers certaines molécules telles que les organophosphorés et les carbamates.

Ainsi donc, nous disposons maintenant d'un certain nombre d'outils biologiques pour apprécier les qualités physico-chimiques et chimiques des systèmes estuariens et portuaires régionaux : outils classiques réclamant une bonne connaissance des biocénoses (espèces indicatrices et bioindicateurs de pollution) et outils dont l'utilisation est actuellement en voie de développement (biomarqueurs variés dont l'emploi devra être élargi à d'autres taxons mais dont la parfaite maîtrise passe par la connaissance de la génétique des populations testées).

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HEAVY METALS IN SEDIMENTS FROM LAGOS LAGOON, NIGERIA

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Lagos Lagoon obtains most of the effluents from industrial, domestic and other anthropogenic sources. This study was designed to monitor the degree of metal contamination of the lagoon through the analysis of sediments, water and biota.

Surficial sediments from the Lagos lagoon have been analysed for their total (HNO_3 + HCL) and exchangeable (IM $MgCl_2$) heavy metal (Cu, Zn, Ni, Cr, Pb, Cd, Co, Ag, V and Mn) concentrations by ICP-AES, EDXRF and AAS. The highest concentrations of the metals in lagoonal sediment were: (i) HNO_3 + KCL-leachates (exchangeable): Cu-16, Cr-126, Ni-76, Zn-262 and Mn-411 ppm. The mean total concentrations of the metals and their exchangeable metal concentrations are high when compared to the metal concentrations in other lagoonal estaurine sediments.

Although, the levels of metals in the water are very low (Cu, Ni, Pb and Cr were 24, 11.0, 33 and 28.4 μgl^{-1} respectively, Zn was 0.23 ppm). The animals (Sepia, Saccostrea and Tympanostomus) contain higher levels than did the water (Cu 1.5 ppm, Pb, 2.5 ppm and Zn, 7 ppm). The levels of the toxic metals are of the order: Sediment > animal > water. This indicates that these aquatic animals can be used along with sediments as indicators of lagoon pollution.

ASSESSMENT OF CHEMICAL SPECIATION OF ZINC AND CADMIUM USING *EICHHORNIA CRASSIPES*

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Chemical speciation of metals in an aqueous media are usually examined using physico-chemical techniques. In this study, however, we have used an aquatic macrophyte as the test organism in order to evaluate factors affecting uptake of two metals, zinc and cadmium by the plant using laboratory bioassay studies. Results indicate that the rate of Zn uptake was influenced by factors such as time of exposure, concentrations of Zn, solution pH, temperature and the presence of EDTA as well as that of a competing cation, Cd. Exposure times of 1-4 h and a test solution concentration of 250 mg/L were found to be optimum for Zn absorption by the plant. Using EDTA as the model ligand to maintain specific concentrations of free Zn^{2+} in solution based on thermodynamic constants, absorption of Zn by plant roots, petioles and leaves were monitored. It was found that Zn uptake decreased with increasing concentrations of EDTA in solution indicating the role of organic complexation in controlling Zn^{2+} speciation in solution. Maximum plant uptake was observed at 35C, while extremes in solution pH also decreased metal absorption. Cd ions exerted a negative influence on Zn absorption and translocation in all plant parts probably due to competition at uptake sites.

L'UTILISATION DE QUELQUES ESPECES DE POISSON POUR UNE CARTE BIOINDICATIVE DE LA POLLUTION D'EAU EN METAUX LOURDS

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Le problème du biomonitoring (observation et recherche biologique) de l'environnement dans les pays de l'ex-URSS occupe une importance particulière dans ces dix dernières années. Cependant, le point sur l'état actuel de la biote, la définition de la tendance de sa transformation, en particulier dans les régions de technologie lourde. L'élément important du biomonitoring est le choix du type d'indicateur. Dans l'écosystème aquatique comme l'écosystème terrestre, le type d'indicateur doit être objectivement et adéquatement réfléter le niveau et l'intensité de l'anthropogenèse dans une recherche donnée accomplissant selon une donnée du Comité de Sciences de Technologie de l'Etat d'Ukraine pour un but d'indication biologique une sélection d'espèces était faite, celle sélection est le fond pour l'ichtyologie de la réserve hydraulique de Zaporogie et enregistrant dans les zones des actions technologiques intenses. Les espèces sélectionnées se distinguent par : la particularité de leur nutrition, de leur comportement et d'autres aspects. Sans oublier leur valeur économique l'on site les espèces industrielles telles que le gardon, du latin (*Rutilus rutilus*) le chabot (*Neogobius fluviatilus*) et le *Rhodeus sericeus* les recherches ont été faites sur la particularité de l'accumulation des métaux lourds par la méthode d'absorption spectrophotométrique atomique. La contenance en zinc de ces espèces atteint des indices maximaux chez les poissons parvenant des endroits où se trouvent des barrages hydrauliques (des eaux basses et hautes de la réserve hydraulique). La contenance en fer, en cuivre, en cadmium et en plomb est plus élevée dans le biotope des endroits moyens des hautes eaux, vers lesquels se déversent les eaux stagnantes industrielles des entreprises métallurgiques et chimiques. Des endroits donnés de l'espace vital sont les pollués des métaux lourds. Les éléments toxiques les plus spécifiquement accumulés sont : le plomb et le cadmium. La contenance en plomb varie en une limite insuffisante chez les poissons de différents endroits, des hautes eaux de la réserve hydraulique, c'est-à-dire elle porte un caractère uniforme. La contenance en cadmium atteint le maximum dans les régions où se déversent les eaux industrielles stagnantes, ensuite la contenance en cadmium graduellement s'abaisse atteint le maximum dans la partie avale de la réserve hydraulique. Le caractère d'accumulation par d'autres espèces des poissons considérablement se différencie des exemples cités ci-dessus.

L'accumulation des métaux dans l'organisme du chabot (*Neogenius fluvialus*) permet de pointer le niveau de pollution d'une couche d'eau donnée. Quant au coefficient obtenu pour le *Rhodeus sereceus* porte un caractère disperse et varie ce qui ne permet pas d'emmener une pointe adéquate du niveau de pollution, c'est ainsi que les données obtenues selon le caractère et le niveau d'accumulation par l'organisme des poissons, des métaux lourds ont permis de mettre au point un système bioindicateur mis sur la carte. La réserve hydraulique de Zaporogie de la région de Dnepropetrovsk et les recommandations en utilisation des espèces pour ces buts, toutefois doivent obligatoirement considérer comme âgé.

Dans n'importe quel cas doivent être considéré comme âgé c'est ainsi que le plus important indice physiologo-biochimique de chaque individu de l'espèce de la faune ichtyologique proposant dans un but bioindicatif et des cartes bioindicatives de n'importe quel écosystème aquatique.

Les tests de transplantation révèlent une augmentation significative des teneurs métalliques chez les gastéropodes transplantés dans la station avale (K2). Ceux transplantés dans la station amont (K1) sont décédés après une semaine d'exposition.

Les résultats des tests d'accumulation sur 7 jours réalisés au Laboratoire sont notables surtout pour le Cd pour lequel on observe des différences significatives dépendant du mode de traitement et montrant l'importance de la forme dissoute du Cd dans la station K1 et de la forme particulaire dans la station K2.

Les extraits de sédiment de la station K1 ne sont pas toxiques envers *D. magna*. Au contraire, une mortalité de 100% est notée après 48 h d'exposition des daphnies à 40% de l'extrait de la station K2.

L'étude de la spéciation métallique dans les sédiments montre d'une part une bonne concordance entre la somme des teneurs en métal de chaque fraction et celle obtenue après minéralisation. D'autre part, la fraction résiduelle au niveau des deux stations demeure importante pour le Zn. En tenant compte du pourcentage des différents métaux dans les diverses fractions des échantillons, la capacité de relargage des métaux dans les eaux peut être établie de la manière suivante :

- sédiment de la station K1 : $Cd > Cu > Zn$
- sédiment de la station K2 : $Cd \approx Cu > Zn$.

Ceci permet de conclure quant au risque de contamination des eaux.

L'ensemble des résultats montre l'importance de faire varier les méthodes d'investigation dans la détermination de la biodisponibilité des métaux dans un milieu aquatique.

La présence en permanence de *Melanopsis praemorsa* dans les deux stations de notre système montre que ce gastéropode est un bon indicateur de pollution métallique.

HEAVY METAL ACCUMULATION IN LEECHS AND SNAILS OF THE RIVER ELBE
- PRELIMINARY RESULTS -

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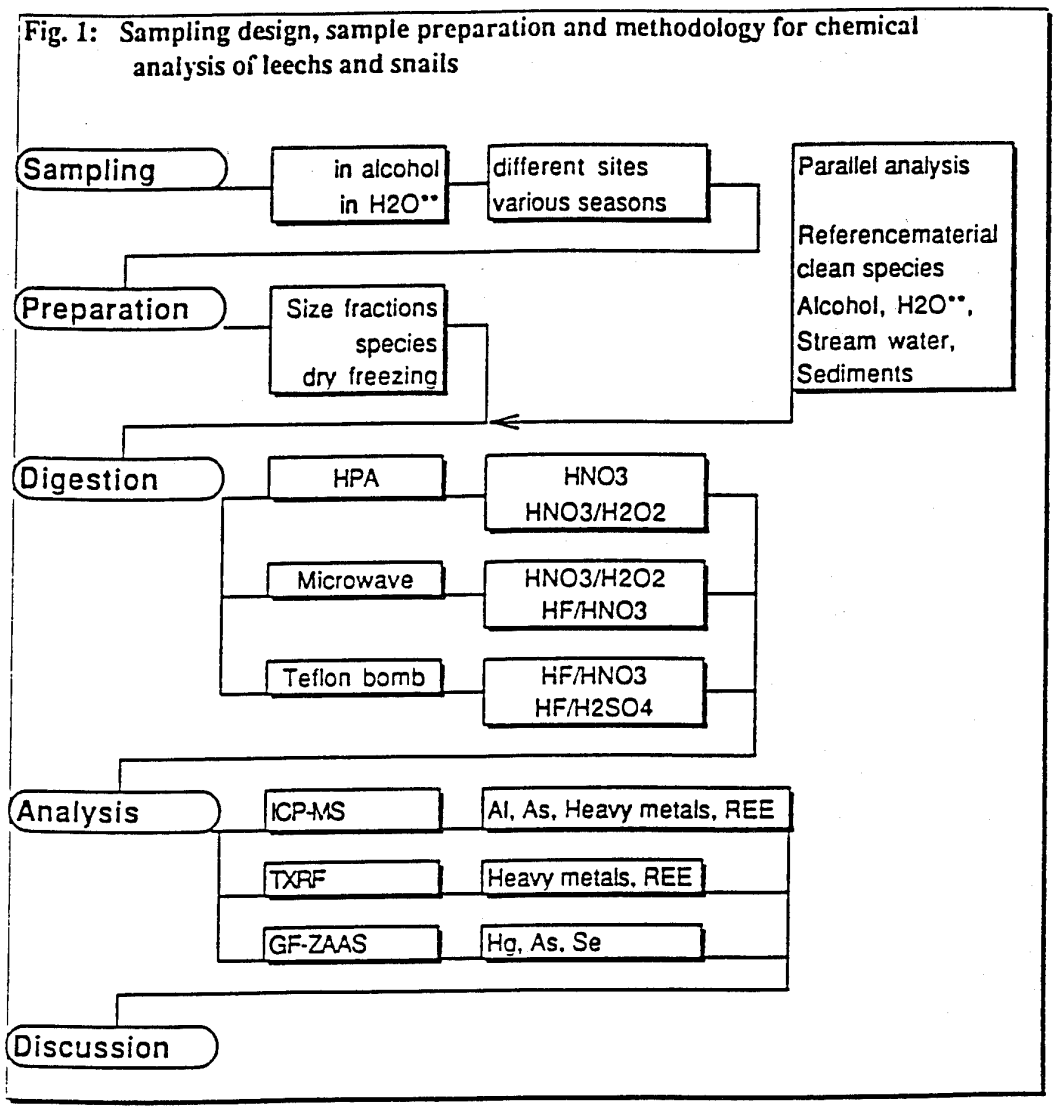
The Elbe river with a catchment area of 700 km^2 is the most polluted stream in Germany. Since the fall of the border in 1990 most of the formerly point sources for contamination like the chemical industry were closed and the water quality improves in general. Nevertheless high concentrations of heavy metals and other toxic elements, organic pollutants and nutrients (N, P) are obvious. Heavy metals and elements like Hg, As, Al are related to the suspended particulate matter (SPM) and are supplied to the Elbe by high contaminated tributaries in Germany and the CSFR.

With the growing water quality of the Elbe a benthic organism symbosis was reestablished. These consist mainly of mikrobenthos like bacteria, algae and protozoa and makrobenthos like Asellus, Erpobdella, Glossiphonia, Radix or Bryozoa (GUHR *et al.* 1993) which in general are settling on stones and other hard substrate. As a result of the higher concentrations of toxic pollutants in the sediments and SPM no benthic organism is visible in the muddy environment at this time (DREYER, pers. com.).

We choose *Erpobdella octoculata* and *Radix ovata* for a study of their bioaccumulation of heavy metals considering different sites (points of pollution) and various discharges of the river at two seasons. The work is orientated more methodological under the aspect of various sampling, digestion and analytical methods. *E.o.* is divided into different size fractions whereas for *R.o* some sub-species are considered. Wet digestion is done by High Pressure Ashing (HPA) as well as by microwave techniques including different acid-combinations. Analyses of heavy metals by AAS, ICP-MS and TXRF will prove the best analytical method for this kind of bio-material. Fig. 1 gives an overview for the proposed investigation. Parallel analysis of water and sediment as well as data of monitor programs are the basis for the interpretation.

Preliminary results e.g. high Cd and Zn concentrations in *E.o.* as detected at this time by AAS and TXRF techniques, respectively will be discussed with respect to different hydrological situations including time and space and in terms of bioaccumulation and biodiversity.

Fig. 1: Sampling design, sample preparation and methodology for chemical analysis of leechs and snails



THE RELEASE OF BIOAVAILABLE HEAVY METALS FROM THE CONTAMINATED MARINE SEDIMENTS: A MONITORING BY A BIOACCUMULATING BIVALVE

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Heavy metals are present in the marine sediments both naturally or bound to anthropogenic contributions to them and the physico chemical and biological behaviour in the bottoms is responsible for the natural processes of transformation, mobilisation and dissolution of the metals in the salt solution.

The "bioavailable" fraction of the total metal from the sediments interact with the organisms, enter and circulate in the internal medium with possible toxic effects. The "bioaccumulating" species detoxify the heavy metals surplus by their immobilisation in target organs, tissues or cellular organelles. Therefore, the measure of the metal content in these tolerant organisms may supply an indication for the presence of bioavailable metals in the ecosystem. At this regard soft bottoms bivalves seem very useful. They filter the water at the sediment interface and their body concentrations reflect the metal fluxing from the sediments.

A study on heavy metal (Cu, Cr, Mn; Zn) bioaccumulation in the bivalve *Corbula gibba*, that is largely dominant the macrofauna from the soft bottoms has been performed in a gas drilling field located offshore Ravenna (Italy) in the Northern Adriatic Sea, contaminated from the discharges of cuttings and drilling muds. The measures of the metal concentration in the soft parts has been performed on *Corbula* from two sampling surveys during drilling and from one survey performed 10 months after the end of drilling.

The data has been evaluated taking into account the biological parameters that may influence the bioaccumulation during the life cycle, i.e. sex, age, biological condition with particular connection to the reproduction processes. Metal levels were tested for the increasing distance from the source of pollution and for the survey sequence. In this comparative approach a transformation of metal levels from µgMe/g soft part dry weight to µgMe(soft part)/g shell has been made in order to avoid the influence of the seasonal changes in the biological condition and the size parameter (weight of the shell) has been considered as covariant.

The results show the control sampling sites, farther than 1 Km from the source of contamination, having constant values of the metals, and the only Cr and Zn in the second period linked to the distance. The metals generally trend from quite high values in the first period (Feb. 1986) to a minimum in the second (September 1986) and then to an increase in the last survey of June 1987 without reaching the levels of the first period. For a better evaluation of the environmental impact of contamination a longer time seem requested in the monitoring studies.

ETUDE DE LA CONTAMINATION METALLIQUE DES SEDIMENTS DE L'OUED BOUFEKRANE (MAROC) : APPROCHES ANALYTIQUE ET ECOTOXICOLOGIQUE

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L'Oued Boufekrane est un cours d'eau permanent dont les eaux sont utilisées à des fins agricoles. Cette rivière reçoit les eaux usées domestique et industrielle de la ville de Meknès (Maroc) sans traitement préalable ce qui engendre une pollution minérale et organique intense (Saadallah, 1991). Le présent travail a pour objectif la comparaison de deux méthodes permettant d'étudier la qualité des sédiments de ce cours d'eau; l'une analytique et l'autre écotoxicologique en réalisant des biotests avec les sédiments.

Dans la partie analytique, les sédiments récoltés de six stations sont séchés à 75°C et minéralisés par attaque acide à l'eau régale et à chaud. Sept métaux lourds (Al, Cr, Cu, Mn, Ni, Pb et Zn) sont analysés dans la fraction inférieure à 80 et 50 µm, au spectrophotomètre d'absorption atomique (Type Philips, modèle Pye Unicam 90.000).

Les caractéristiques physico-chimiques des sédiments sont également déterminées.

Dans les biotests nous avons utilisé le matériel suivant:

a- Les sédiments : le prélèvement, la conservation et la préparation des extraits de sédiments sont effectués suivant les recommendations de l'ASTM (1991). Seule la fraction inférieure à 63 µm est retenue.

b- Deux crustacés d'eau douce obtenus à partir d'un élevage au laboratoire :

- *Gammarus gauthieri* âgé de moins de sept jours. Ce test est pratiqué sur les extraits aqueux et sur la phase solide des sédiments (contact direct) suivant les protocoles standards décrits par Nelson *et al.* (1989). Après 10 jours d'exposition on détermine la CL50 (10 j).

- *Daphnia magna* âgé de moins de 24 heures. Ce test est réalisé uniquement sur les extraits aqueux des sédiments suivant les recommendations de l'OCDE (1984). Après 48 heures d'exposition on détermine la CL50 (48 h).

Les résultats analytiques montrent les observations suivantes :

- La répartition des stations selon l'axe horizontal illustre bien l'opposition amont-aval. Cette opposition est expliquée par les variables Cu, Pb et Zn pour les stations avales et par le Mn pour les stations amonts.

- Les valeurs révélées pour ces métaux (Cu, Pb et Zn) sont largement supérieures au fond géochimique du bassin versant ce qui explique un apport anthropique.

- Les facteurs d'enrichissement relatifs au Cu, Pb et Zn croissent régulièrement pour atteindre 30, 12 et 2 respectivement pour le Pb, Zn et Cu dans les stations avales.

- Les concentrations des autres éléments (Al, Cr et Ni) dans les sédiments sont nettement moins accusées.

- Les indices de pollution moyens permettent de classer par ordre décroissant les stations selon leur degré de contamination suivant :

$5 > 4 > 6 > 2 > 1 > 3$

- L'étude des associations géochimiques existant entre les métaux analysés et les paramètres physico-chimiques des sédiments montre que certains éléments métalliques semblent s'associer soit à l'un, soit à l'autre des substrats.

EVALUATION DE LA BIODISPONIBILITE DES METAUX LOURDS DANS UN ECOSYSTEME SOUTERRAIN (KHETTARA)
- APPLICATION DE DIFFERENTES APPROCHES ECOTOXICOLOGIQUES -

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Ce travail entre dans le cadre d'une action intégrée franco-marocaine dont l'objectif est l'étude de la contamination par les métaux lourds des écosystèmes terrestre et aquatique du champs d'épandage des eaux usées de la ville de Marrakech.

Cette étude s'intéresse aux résultats concernant un écosytème d'eau douce particulier appelé « Khettara » (système d'irrigation par drainage de la nappe phréatique). Elle fait suite à des recherches précédentes (MAZLANI *et al.*, 1994), qui ont montré la contamination par le Zn, Cu et Cd des matières en suspension (MES), des sédiments, de la spyrogyre et d'un gastéropode prosobranche (*Melanopsis praemorsa*) de ce système. Cependant, contrairement aux résultats dégagés à partir des MES et des sédiments, les concentrations métalliques trouvées chez la spyrogyre et les Mélanopsis sont plus élevées dans la station amont (K1) que dans la station avale (K2) de ce système.

Au vue de ces données, l'évaluation de la biodisponibilité métallique dans cet écosystème par différentes approches écotoxicologiques s'avère nécessaire et pourrait éclaircir ce phénomène. Pour cela on fait appel dans cette étude à :

- Des tests d'accumulation *in situ* : Des Melanopsis récoltés d'une station témoin située hors du champ d'épandage des eaux usées sont transplantés aux stations amont (K1) et avale (K2) de notre système. Ces tests sont réalisés sur une période de trois semaines dans des cages grillagées et plastifiées.

- Des tests d'accumulation au laboratoire : ils sont effectués sur des Melanopsis temoins en utilisant des eaux traitées en provenance des stations K1 et K2. Le traitement consiste à la filtration sur des millipores (0,45 µm) et à l'ajout de 40% de l'eau d'égout filtrée ou non filtrée.

- Des tests de toxicité des extraits de sédiments. Ces extraits sont obtenus suivant le protocole standard décrit dans l'ASTM (1991), et les tests sur *Daphnia magna* sont effectués selon la méthode standard préconisée par l'OCDE (1984).

- L'étude de la spéciation métallique dans les stations K1 et K2. Celle-ci est faite par extraction sélective en suivant le schéma préconisé par CARRE et WELTE (1986).

La partie molle des gastéropodes est séchée puis minéralisée par attaque acide. Les analyses sont effectuées au moyen de la spectrophotométrie d'absorption atomique (modèle Varian 475-AA) à flamme pour le Zinc et le Cuivre et au four pour le Cadmium.

Les résultats de la deuxième partie ont permis de classer par ordre décroissant le degré de toxicité des sédiments étudiés. Ce classement varie légèrement en fonction des tests mais les deux modes d'exposition des gammares aux sédiments confirment le même classement :

6 > 5 > 3 > 2 > 1 > 4 selon le test *G. gauthieri*

5 > 6 > 3 > 2 > 1 > 4 selon le test *D. magna*

Ces classement montrent différents types de stations qui diffèrent par la qualité de leurs sédiments et par le degré de leurs toxicités.

Les données obtenues pour la station 3 et 4 permettent de discuter l'intérêt et les limites de la méthode des biotests. Cette méthode permet d'évaluer la toxicité des polluants hydrosolubles biodisponibles dans nos conditions expérimentales d'extraction et d'exposition mais demeure une technique complémentaire à celle des analyses physico-chimiques.

HEAVY METAL POLLUTION IN A LAGOON IN THE SOUTHERN REGION OF SRI LANKA

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Aquatic systems in Sri Lanka are subjected to pollution due to the haphazard use of pesticides, weedicides and also due to natural processes and these pollutants may enter edible plants and animals living in such systems. Fish is the major source of protein for most Sri Lankans and therefore it is very important to monitor the levels of pollutants present in aquatic systems as detailed studies on such aspects are not reported in Sri Lanka. Present work includes monitoring of heavy metal pollutants in a lagoon in Sri Lanka and attempts were made to identify indicator organisms for aquatic pollution.

Dondra lagoon which is situated in the southern tip of Sri Lanka was selected for the present study and three zones within the lagoon, viz. close to the sea, middle of the laggon and end of the lagoon towards the land, were selected for sampling. From each zone water samples from different depths were collected once a month, sediment samples were collected once in two months and samples of plankton, crabs and fish (*Etroplus suratensis*, *Oreochromis mossambicus*, *Macronus vitatus*, *Thrissocles mystax*, *Gerris abbreviatus* and *Hemiramphus gaimardi*) were also collected depending on their availability. Different tissues and organs (exoskeleton, fins, scales, skin, gills, flesh, bones, eyes, liver, gonads, viscera, fat-bodies and stomach contents) of edible fish were separated before subjected to analyses. All the samples were analysed for Zn, Cu and Pb by Atomic Absorption Spectroscopy. Precautions were taken to minimize possible contaminations during collection, extraction and analysis of samples. Extraction of samples was carried out according to the method outlined by Anderson (1) which was modified during the present study.

High concentrations of Pb were found in zones close to motor ways and high concentrations of Cu and Zn were found in zones close to paddy fields, coconut husk pits and areas where human activities are common, when compared to other zones in the lagoon. Possible sources of pollutants in each zone and the chemistry and physical factors involved are discussed. Concentration of Zn was found to be high in fins and scales of most fish and in eyes of *Thrissocle mystax*. High concentrations of Cu were observed in the liver of *Oreochromis mossambicus*. High concentrations of Pb were found in all the tissues of *Etroplus suratensis* and in some of the tissues in crabs. Furthermore, effect of feeding habits, position in the food chain and tissues of living organisms in which heavy metals are accumulated etc. are discussed.

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METAL DISTRIBUTION IN A SUB-TROPICAL COASTAL LAGOON: A STATISTICAL APPROACH

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The problem of metal distribution in sediments from coastal environments has largely been studied (e.g.: Bevan *et al.*, 1975; Brinckman & Iverson, 1975). From this kind of approach arises serious interpretation problems, since, fraquently a large number of samples are to be collected as well as a large amount of variables are considered. The use of concentration maps has been widespread, but, from then it is quite difficult to see relations between variables and no behavior inference is possible to be taken. The use of statistical tools in order to clear up behavior of metals as related to other variables is suggested (Vernette, 1970).

In this study, 56 sediment samples were collected in Guarapina lagoon, a shallow subtropical system close to Rio de Janeiro, Brazil (choked lagoon; Kjerfve, 1986; figure 1), which were analysed for Particulate Organic Carbon content (POC), % of silt-clay content (%<63μ) and the major elements Se, Al, Fe, Mn, Mg, Ca, Ti, K, Na, and the trace metals Pb, Cu, Zn, Ni, Zr, Cr, Sr, and Ba, by x-ray Fluorescence. The choice of statistical tools is related to the kind of available data and type of informations are to be obtained. Considering the amount of available data in this work Principal Components Analysis (PCA) was chosen (Vernette, 1970; Dagnelie, 1975). Results are presented in table 1 and 2.

River PCA: Zn, Ni, Mn and Cr are positively related to the major elements Ti, Fe, Al, Ca, and Mg and to silt clay fraction and POC under factor I. This is due to the mixed composition of the riverine sediments, composed of detritic feld-spars, ferromagnesians and products of leaching, such as kaolinites and Fe-hydroxides. Si and Pb are also related in this factor I, but negatively. Pb enrichment in riverine sediments is due to its association to detritic minerals as well as concentration of this element as related to other more labile elements. Factor II associates positively Cu, POC and clay-silt what is the result of the quite low concentraitons of these variables in the river sediment. On the other hand, Pb, Zr, Sr, and Ba are negatively associated to K and Na characteristic compounds in fledspars. Factor III show that Zr and Na are associated to detritic coarse sediments as in Factor II, but these two elements are individualized here, since they are main compounds in Zircon and of marine origin, respectively.

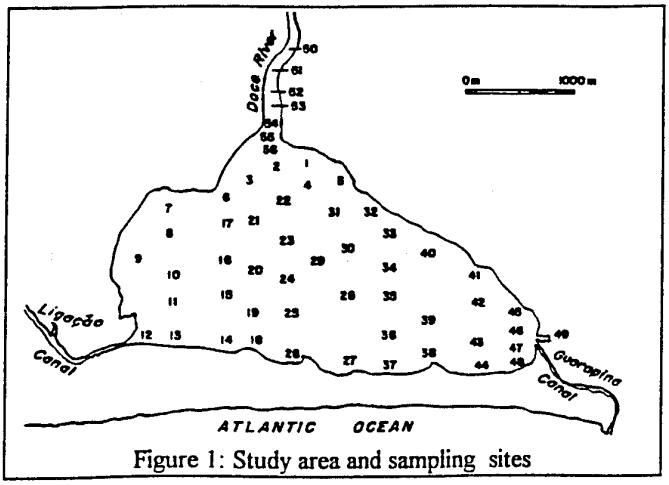

Figure 1: Study area and sampling sites

Lagoon PCA: Factor I put together most of the studied variables (table 1). Among the elements present in factor I, silicium is the only negatively correlated, what is due to its very low reactivity. Factor II negatively associates Ba, Zr and K, elements associated to the primary detritic minerals (feldspars, quartz and mica; Wasserman, 1991) that are not dominant in the lagoon. The factor III characterizes Cu behavior as different of all other elements. This could be explained by association with immature organic matter. The results show that the lagoone is a preferential site for precipitation of material originated from the drainage basin decomposing rocks. Most of the elements that are leached from the continental alteration profiles are subjected to the influence of the organic matter and silt-clay particles being trapped during its transport. However, in the brackish waters of the lagoon, the hydrodynamic conditions seem favour this trapping.

The above results seem to separate the elements in three groups following their behavior. The first group is characterized by association with POC and clay-silt fraction. Fe, Zn, and other trace elements are in this group. The second group is represented by the elements associated to coarse detritic minerals and its decomposition product kaolinite (trapping important amounts of Sr). Ba, K, Na, and Sr are among the representatives of this group. The third group is represented by Cu, that is associated with organic matter.

Further studies on elemental geochemistry in Guarapina lagoon should focus on Fe, Cu, Zn and Sr as representatives of the groups depicted by statistical methodology presented in this work.

	FACTOR I	FACTOR II	FACTOR III
+	Na, Ca, Sr, Al, Fe, Mn, Ti, Cr, Zn, Ni, Pb, Mg, POC, %<63μ		Cu
-	Si	Ba, Zr, K	

Table I: Schematic presentation of PCA for the lagoon

	FACTOR I	FACTOR II	FACTOR III
+	Zn, Ni, Mn, Cr, Ca, Mg, Ti, Fe, Al, POC, %<63μ	Cu POC, %<63μ	Zr, Na
-	Pb Si	Pb, Zr, Sr, Ba, K, Na	

Table II: Schematic presentation of PCA for the river

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SYMPOSIUM B3

CADMIUM CYCLING IN SHEEP - GRAZED HILL COUNTRY PASTURES

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Background

The accumulation of cadmium (Cd) in the agricultural environment increases the potential for Cd to enter food products and ultimately affect human health. Continual exposure to small amounts of Cd present in the pastoral environment often leads to an accumulation, by grazing animals, in tissues such as the liver and kidneys. For example, over the years 1988 - 1991, some 22-28% sheep slaughtered at export killing facilities exceeded the maximum residue level (MRL) of 1 ppm Cd in meat and offal for human consumption.

The potential threat, to New Zealand's export meat trade, of Cd being used as a non-tariff trade barrier has resulted in the urgent need to know more about Cd accumulation in pastoral farming, the important sources of Cd and the factors affecting the availability and ingestion of Cd by livestock.

This paper reports on a farmlet study, now in the second year, which was initiated to monitor seasonal variations in soil and pasture Cd status as influenced by rate of phosphatic fertiliser application and paddock strata, as well as determine the effect of two different stocking rates on Cd accumulation in sheep tissues.

Approach

In March 1993, a two farmlet study was established on hill country, ranging from gentle (<10°) to very steep slopes (>40°). The soils are sedimentary (yellow-brown earths) and pastures were established around 20 years ago. Since 1979 the study area has been a phosphate (P) fertiliser trial with 5 rates of application *viz:* 10, 20, 30, 50 and 100 kg P/ha, applied in autumn, either as single superphosphate (1979-1990) or triple superphosphate (since 1990). The trial area comprises two blocks (7 ha/block) of 5 paddocks each, with each paddock receiving one of the fertiliser rates. Sheep flocks were rotationally grazed over the 5 paddocks within a block at either 11 or 16 stock units (su)/ha representing lax and hard grazing pressure.

Each paddock is divided into 4 different sampling strata *viz:* steep slopes (30-40°), easy slopes (10-20°), stock campsites and tracks. Soil (0-7.5 cm) and herbage (mixed pasture) samples are collected every 28 days from each strata within each paddock. Faecal samples are also collected every 28 days from 10 randomnly selected ewes in each flock to estimate geophagy (soil ingestion) using faecal titanium analysis. Ten randomnly selected sheep from each flock, from the nucleus flocks, are slaughtered each year at the trial anniversary to enable tissue sampling for Cd analysis.

Analytical techniques include acid digestion followed by flame or graphite furnace atomic absorption spectroscopy.

Results

Interim results suggest that the average annual Cd concentration for soils and pastures was 0.24 ppm and 0.25 ppm respectively although the variation between samplings was quite large i.e.,

0.15 - 0.42 ppm. Total soil Cd values reflected soil P status and pasture Cd values reflected total soil Cd values. Significant differences in Cd content of both soils and pastures occurred depending on paddock strata sampled. Highest soil Cd concentration occurred in campsites (0.36 ppm) where paradoxically the lowest herbage Cd (0.15 ppm) occurred. Conversely, the steep slopes showed the lowest soil Cd concentration (0.15 ppm) but the highest herbage Cd concentration (0.40 ppm). Extractable Cd (in 0.05 M $CaCl_2$) was 30% and 49% of the total Cd on campsites and steep slopes respectively. Easy and track areas were intermediate between the two extremes.

Faecal Cd concentrations were generally lower for the lax grazed flock (0.54 ppm Cd) than for the hard grazed flock (0.73 ppm Cd) while the annual amount of soil ingested was calculated as 14 and 20 kg soil (assuming 75 and 70% pasture digestibility) for the lax and hard grazed flocks respectively. Estimates of Cd intake by the sheep in the current trial showed that relatively insignificant amounts of Cd was ingested by the lax (2%) and hard (4%) grazed sheep through soil ingestion.

Grazing management (lax vs hard) had little effect on the retention of Cd in sheep tissues with muscle tissues containing 0.01 ppm Cd, liver tissue 0.43 ppm Cd and kidney 1.05 ppm Cd on average. Kidney Cd concentrations increased 0.15 ppm in the first year of the trial.

LEAD AND CADMIUM EXCRETION THROUGH MILK IN THE EWE

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Lead and cadmium are cumulative toxics in the continental trophic chain. Our study aims at assessing the risk of contamination of foodstuffs of animal origin for humans by these two metals. The selected model was the ewe.

After studying lead excretion through milk in cattle (1) and cadmium pharmacokinetics in the non lactating ewe (2), we have now started the study of pharmacokinetics and lead and cadmium excretion through milk in the lactating ewe.

Twenty-four Prealp ewes, aged from 2 to 6 years were alloted to 5 groups. A control group of 5 animals received either lead alone or cadmium alone or both lead and cadmium, with or without zinc throughout the lactating period (52 days). They received 2.3 mg/kg/d lead as chloride, 2.5 mg/kg/d cadmium as chloride over a 21-day period, then 1.5 mg/kg/d cadmium as chloride over a 31-day period, 3.5 mg/kg/d zinc as oxide. The diet consisted in dehydrated alfalfa granules, oats, hay and vitamined mineral additive.

Blood samplings for dosing heavy metals were performed until day 133. In this account we shall only consider those performed on the days of milk samplings: days, 0, 7, 14, 21, 28, 35, 42 and 49.

The measurements of metals in the blood and in the milk were performed by atomic absorption spectrophotometry with L'VOV platform graphite furnace after 1/10th dilution in triton X at 0.5% and nitric acide 0.05N. Detection limits are 4 µg/l for lead and 0.3 µg/l for cadmium.

Lead values rise quickly in all the groups but very differently in each animal. When compared, the average lead values in each group are rather close 127±35 µg/l in group Pb, 113±49 µg/l in group Pb-Cd, 120±28 µg/l in group Pb-Cd-Zn and values inferior or equal to the detection limit (4 µg/) in the cadmium group and the control group.

Cadmium values reach a steady state after 14 days. In the cadmium group, the average is 1.6 µg/l ±0.6 with few differences between animals. In the group Pb-Cd, the average is 2.7 µg/l ±1.6, with 3 animals whose averages range from 4.7 µg/l to 3.1 µg/l. In group Pb-Cd-Zn, the average is 2.2±1.1 µg/l, with 1 animal whose average is 4.4 µg/l.

Lead concentrations in milk stabilizes quickly. The animals with the highest lead values are those whose concentrations in milk are the highest. The average values of the steady state in milk are higher than the average values of the steady state in blood. The ratios mild concentration/blood concentration range between 1.5 and 2 (x=1.72±0.4).

Therefore, lead and cadmium whose concentrations in milk are higher than concentrations in blood are subjected to a process of active secretion in the udder.

Yet the coefficient of transfer i.e. the ratio between the amount excreted through milk and the amount administered is very low - close to 0.1% for lead and 0.005% for cadmium.

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TRANSFER OF RADIOCAESIUM, PLUTONIUM AND AMERICIUM TO SHEEP AFTER INGESTION OF CONTAMINATED SOIL

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Ingestion of contaminated soil has been identified as a possible mechanism for transfer of radionuclides to ruminant animals and ultimately via the foodchain to humans. Soil ingestion will be particularly important when radioactive half-lives are long and the transfer from soil to pasture vegetation is small. A determination of the contribution from soil ingestion to the intake of radionuclides is relatively straightforward, and so this process has been studied by various workers. In contrast, the availability of the ingested activity for uptake is more difficult to assess, particularly if *in-vivo* experiments are involved, and so this aspect has received much less research attention.

A simple small scale *in-vitro* procedure has been developed for assessing the potential bioavailability of some radiologically significant radionuclides when ingested with soil (Cooke *et al.*, 1995). Values obtained for radiocaesium agreed well with measurements of absorption by ruminants obtained by other workers. Values obtained for actinide elements were in excess of measurements of absorption across the gut made elsewhere. The results were however consistent with the reduced availability of soil-associated activity observed in limited *in-vivo* studies, compared to that when the activity was administered in a soluble form.

An *in-vivo* experiment has therefore been undertaken to validate the *in-vitro* approach. Absorption of radiocaesium was estimated using a dual isotope technique (^{134}Cs administered intravenously as chloride; ^{137}Cs administered orally as contaminated soil). The true absorption coefficient (A_t) was calculated from the ratio of the isotopes in tissues or urine relative to the rates of administration. Simultaneously the gut absorption factor (F_1) of $^{239/240}Pu$ and ^{241}Am from the contaminated soils was estimated from activity in tissues at the end of the experimental period.

Two soils were administered mixed with the diet to the experimental sheep: an alluvial gley from a tidally washed pasture contaminated by discharges from the Sellafield Reprocessing Plant, and a fen peat artificially contaminated in a lysimeter at the National Radiological Protection Board. Simultaneously an infusate containing ^{134}Cs was administered intravenously through a jugular catheter. Each soil treatment was administered to a group of four sheep. A third group of sheep was used as a control. Administration of soil and infusate was continuous for a period of four weeks, during which samples of faeces and urine were collected daily. At the end of the experimental period animals were slaughtered and tissue samples taken for analysis.

True absorption measurements of radiocaesium were found to agree well with estimates made *in-vitro*. A_t from the alluvial gley soil was found to be > 0.05. The latter is a much lower availability than previously found with other peat soils. However the peat used was a very different soil to the organic soils which have been found to fix radiocaesium inadequately elsewhere. Gut absorption of plutonium and americium were found to be lower than from the *in-vitro* procedure. Transfer from the two soils was not found to be significantly different. Is is therefore suggested that the higher actinide availability inferred in vitro is due to association with high molecular weight species that give complexes that are soluble *in vitro* but are not absorbed *in vivo*. These species could be either colloids or long chain organic molecules, such as humic substances originating from the soil.

The results obtained suggest that for radiocaesium, ingestion of contaminated soil would under normal circumstances provide only a small proportion of the total radiocaesium burden of the

animal. For actinide elements ingestion of contaminated soil will provide a significant proportion of the animal's plutonium and americium burden. However the low level of gut absorption means that the absolute quantities absorbed will be much less than would be predicted using current radiological assessment methodology.

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TENEUR ELEVEE EN CADMIUM DANS LA VIANDE DE CHEVAUX POLONAIS : CONSEQUENCES

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Des plans de surveillance renouvelés tous les ans sont réalisés au niveau national afin de contrôler la qualité des denrées d'origine animale destinées à l'alimentation humaine. Ces contrôles sont étendus aux denrées importées. Ces plans sont financés par la Direction Générale de l'Alimentation. Plusieurs d'entre eux sont pilotés techniquement par les laboratoires du CNEVA, les analyses sont réalisées dans les laboratoires vétérinaires départementaux, qui constituent de véritables réseaux par catégorie de contaminant.

L'évaluation des teneurs de contaminants physico-chimiques comme les métaux lourds dans les viandes requièrent des méthodologies très spécifiques pour vérifier la conformité des produits.

En 1992, dans le cadre du plan de surveillance des viandes d'animaux de boucherie, des teneurs de cadmium élevées ont été décelées dans des viandes et des abats de chevaux importés de Pologne vivants et abattus en France.

Le cadmium a une rémanence biologique extrêmement longue (demi vie de 30 ans chez l'homme) et l'organe cible majeur est le rein. Lorsque la concentration du cadmium dans le rein dépass une valeur critique, un trouble fonctionnel irréversible peut évoluer graduellement vers l'insuffisance rénale. Une pathologie osseuse a été observée la première fois en 1950 (maladie Itaï-Itaï) au Japon chez des populations rurales à la suite d'ingestion de riz très fortement contaminé au cadmium...

En Belgique, où la contamination d'origine industrielle est une des plus élevée du monde, des enquêtes ont montré que l'apport alimentaire en cadmium est de 0,22 µg/kg/j. Les principaux vecteurs sont par ordre décroissant : les denrées végétales fruits et légumes (30%), les céréales (16%), les produits laitiers (18,5%) et les viandes et abats (10%). Les viandes et les abats contribuent pour chacun 5% à l'ingestion de cadmium. Il faut tenir compte de grandes différences suivant les habitudes alimentaires.

L'examen des résultats des plans de surveillance dans les produits carnés français donn des teneurs moyennes dans les muscles très basses de 25 à 30 µg/kg alors que des valeurs maximales conseillées par le Conseil Supérieur d'Hygiène public de France sont de 100 µg/kg dans le muscle, de 1 mg/kg dans le foie et de 2 mg/kg pour le rein. Les recommandations de l'Union Européenne ne concerne que le foie (1 mg/kg). La teneur du rein qui est l'organe qui peut fixer 30% de la quantité absorbée n'est pas mentionée.

Ce manque d'harmonisation et l'absence de normes résultent des difficultés à obtenir desrésultats comparables pour les teneurs faibles comme celles mesurées dans le muscle et le lait. Les dosages de cadmium à ces teneurs ne peuvent être réalisée que dans des laboratoires spécialisés, équipés d'appareils très sensibles.

On a observé dans les chevaux français des teneurs en cadmium plus élevées que dans les muscles de bovins sur 35 animaux, 2 avaient des teneurs comprises entre 100 et 500 µg:kg alors que sur 170 chevaux polonais 54 étaient compris entre 100 et 500 mais 18 avaient des teneurs

supérieures à 500 µg/kg dans le muscle. Les valeurs dans le foie dépassant 50 000 µg/kg pour 10% des animaux venant de Pologne.

Un contrôle systématique a été mis en place et les carcasses ont été consignée à l'abattoir attendant les résultats. Ces mesures ont été étendues aux carcasses et viandes importées dont le contrôle est plus difficilement surtout l'origine moins précisée.

L'Union Européenne a été avertie et des garanties ont été demandées aux autorités polonaises :

Les chevaux en provenance des régions les plus contaminées par des activités minières ou industrielles, ne peuvent plus rentrer en France, la pollution du sol dans le cas du cadmium prend une ampleur particulière (Fleischer et coll. 1974). L'âge limite des animaux vivants importés en France a été fixé à 10 ans, le niveau de contamination étant en relation directe avec l'âge de l'animal.

Des contrôles aléatoires sont maintenus pour vérifier le respect des recommandations communautaires. Cependant cet épisode met en évidence l'utilité des plans de surveillance pour repérer les produits qui peuvent augmenter l'apport en substances toxiques. Il est prévu de mettre en place des Limites Maximales de résidus (LMR) pour les métaux lourds et donc le cadmium en application du règlement 93/315/CEE.

Le comité mixte FAO/OMS a fixé depuis 1972 une dose hebdomadaire acceptable de 500 µg pour un adulte, cette dose très basse traduit le caractère cumulatif du cadmium.

THE POTENTIAL PATHWAYS OF PLANT CONTAMINATION IN THE EMISSION REGION OF COPPER FOUNDRY "GLOGÓW"

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Two-year field experiments were carried out to determine to what degree current emission and polluted soil contaminated seven species of plants: corn, hemp, horse-bean, carrot, red beet, lettuce and cabbage.

The plants were grown on the polluted soil in the emission region and on the same soil transported beyond the emission region.

Lead, cadmium, zinc and copper levels in the plants were determined by means of atomic absorption spectrophotometry /AAS/.

The metals concentration was studied both in green parts of the plants and in their roots.

Attempts were made to find out which of the plant species were least contaminated in order to recommend them for cultivation in the ecologically threatened areas.

The studies revealed that plant contamination depended to a great extent on the current emission and not only on the concentration of metals in the soil.

The content of metals, particularly Pb and Cu, in plants grown near the copper foundry was higher than in those cultivated on the same soil transported outside the emission zone.

Stalks, leaves, pods and coat corncobs were more contaminated than seeds and corn. In root crops a particularly high level of metals concentration was found in peels.

The application of ecological, organic-mineral fertilizer "Humidol" on the polluted soil in many cases reduced contamination of plants.

POLLUTION PAR DES METAUX ET DU FLUOR DECOULANT D'ETABLISSEMENTS INDUSTRIELS D'EXTRACTION, DANS UNE AIRE AGRICOLE

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Les exigences d'ordre économique et occupationnel portent souvent à sous-estimer les risques d'altération de l'environnement qui découlent d'une concentration industrielle élevée.

La présence d'éléments traces toxiques dans les sols entraîne des risques pour les animaux et les plantes voies de passage possibles dans les chaînes alimentaires.

L'objectif de ce travail est de connaître la répartition dans le sol et dans la végétation de quelques éléments polluants (Pb, Cd, F) découlant d'établissements industriels pour évaluer la mobilité dans les sols et la biodisponibilité vers les plantes.

Dans 23 sites plantés en vigne, choisis selon la distance du pôle industriel, on a prélevé des échantillons de feuilles, de raisins et de sols pendant cinq ans consécutifs.

On a déterminé les métaux par un spectromètre d'émission (torche à plasma) et le fluor par une électrode spécifique.

Les résultats indiquent une claire relation entre la concentration des éléments polluants, la distance et la position du site par rapport aux établissement industriels.

Le contenu total de plomb et cadmium des échantillons de sol examinés ne dépasse pas le niveau de toxicité, mais l'apport élevé en métal extractible avec EDTA peut être phytotoxique pour la végétation et dangereux pour la qualité des eaux profondes.

Le contenu des éléments polluants dans la végétation est bien au-dessus que les grilles de concentration critiques, avec des valeurs de 4 à 60 fois supérieures dans la cas du plomb.

Les concentrations de plomb et de fluor dans le moût de raisin examiné sont au-dessus des limites établies pour la commercialisation du vin.

FACTORS AFFECTING THE UPTAKE OF LEAD BY CROPS FROM CONTAMINATED SOILS

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The accumulation of lead (Pb) in the environment has long been recognized as a hazard to human health. The potential pathway of Pb from contaminated soils to food crops is not recognized as critical but is nevertheless important and can contribute appreciably to the Pb intake by some communities.

The objective of this paper is to evaluate some of the key soil and environmental/management factors which may influence the absorption of Pb by crops growing on Pb-contaminated soils. The paper reports previously unpublished results from glasshouse experiments and field studies carried out in South Australia.

Glasshouse experiments using six agricultural soils for clover and four for wheat showed that the uptake of Pb by clover and wheat responded linearly with increasing application rates of Pb to 3,000 mg kg^{-1} for clover and about 1600 mg kg^{-1} for wheat plants. Transfer coefficients for clover greatly exceeded those for wheat plants. Uptake of native soil Pb by subterranean clover did not respond to change in soil pH but uptake was negatively related to pH when soils were contaminated by added Pb salts (40 mg Pb kg^{-1} soil). These results generally confirm published reports. Transfer coefficients for Pb uptake by clover varied from about 0.02 to 0.20 [kg (soil)/kg (plant)] between different soils. Addition of phosphate (equivalent to about 80 kg P ha^{-1} to these moderately contaminated soils did not affect the uptake of Pb by clover, in contrast to some published studies of more heavily contaminated soils; nor did applications of nitrogen (equivalent to about 35 gk N ha^{-1}). The effect of placement of added Pb and cadmium (Cd) on crop Cd and Pb concentrations was evaluated using glasshouse-grown subterranean clover plants (about 2.5 kg soil/pot). Whereas uptake of Cd by clover was markedly enhanced when mixed throughout the soil, compared to application to the upper 1 cm surface layer, uptake of added Pb was little affected by its manner of placement. Limited access of contaminant Pb by plant roots must have been compensated for by increased solubility of Pb in the surface layer. The effect of soil temperature on the uptake of native and added Pb (about 50 mg kg^{-1} soil) from two agricultural soils was evaluated in comparison with Cd. Whereas the recovery by plants of soil applied Pb at 10°C was not measurable, about 1% of applied Cd was taken up by clover plants under the same conditions. Uptake of native Pb (from control treatments) was unaffected by temperature increase but absorption of soil applied Pb (abour 50 mg Pb kg^{-1} soil) was at least doubled; recovery of applied Pb was about 0.02%. By comparison, uptake of both native and applied Cd (about 8 mg Cd kg^{-1} soil) increased about three-fold at 18°C compared to 10°C, and % recovery of applied Cd was about 2.5%. Data will also be presented from field studies of pasture and cereal crops in South Australia showing that transfer coefficients of Pb to crops are consistently one to two orders of magnitude less than Cd which in turn are less than zinc. A similar difference in transfer coefficients between Pb and Cd will be presented for vegetables grown on smelter-contaminated soils in home gardens in Pt Piric, South Australia. Even though Pb is only absorbed by vegetables with difficulty, the ease of absorption of soil Pb by different types of vegetables in the Pt Pirie contaminated soils was assessed as follows: silverbeet, carrot, beetroot > rhubarb, lettuce > cabbage, bean, capsicum, potato >> tomato, cucumber, trombone. The occurrence of high Pb concentrations in leafy and root vegetables accords with published work. The high affinity of Pb for soil surfaces makes the

prediction of phytoavailability of Pb by soil tests very difficult. Some of our experiences with soil testing of both urban and rural contaminated-soils for Pb availability is discussed.

ACCUMULATION OF Pb, Cd, AND Zn FROM CONTAMINATED SOIL TO VARIOUS PLANTS AND EVALUATION OF SOIL REMEDIATION WITH INDICATOR PLANT (*Plantago lanceolata* L.)

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Bioavailability of harmful substances in the soil - plant system depends on numerous physical, chemical and biotical processes. Heavy metals as harmful substances are especially important because they accumulate in soils and have a long residence time. Depending on their concentrations these elements can reduce soil fertility and casue an increased input into the food chain, which can endanger the health of animals and humans.

The accumulation of some heavy metals by different plants most commonly used for food, from the soil contaminated with cadmium, lead and zinc are presented in this paper. The vegetables, crops, and the indicator plant narrow leafed plantain (*Plantago landeolata* L.) were used in field experiment in differently polluted areas to determine the uptake of heavy metals (Cd, Zn, Pb) by plants. The highest concentrations of heavy metals were observed in edible green parts of vegetables (endive, spinach), roots (carrot) and tubers (red beat). The heavy metal content in leguminous plants (pods and seeds) was very low compared to high soil concentrations. Tomato and crops (wheat, maize) show lower concentration in fruits and kernels than in green parts.

Soil amendments (lime, vermiculite) were used for the remediation of the heavy metal polluted soil in pot experiment. An indicator plant was used to estimate the effects of the amendments on the uptake of metals by plant. The results showed that the concentration of metals in the indicator plant decreased in the presence of both, lime and vermiculite. This indicates the potential use of those amendments for reduction of metal uptake by plants.

TRANSFER OF Cd, Cu, Ni, Pb and Zn FROM SEWAGE SLUDGE-TREATED SOILS INTO THE EDIBLE PARTS OF WHEAT, CARROTS, AND SPINACH

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Transfer of trace metals from sewage sludge-amended soils into the edible parts of food crops is dependent on several factors, including soil metal concentration, soil pH, soil texture, $CaCO_3$ and species of food plants to be grown. Therefore, the relationship between sludge disposal on agricultural soils and metal accumulation in food crops is very complex. The available information on factors controlling the transfer of trace metals from sludge-amended soils to different food crops has limited field applicability because most of the studies have been conducted by frowing a single crop often using one or two soils.

The studies, reported in this paper, were designed to gain information on factors controlling the accumulation of Cd, Cu, Ni, Pb and Zn in wheat, carrots and spinach grown on 22 (13 sludge-amended and 9 uncontaminated) widely varying soils. The crops were grown in large tubs, each containing ~25 kg air dry soil, kept in the open air.

The results showed that the concentrations of Cd, Ni and Zn in edible parts of wheat, carrots and spinach grown on the sludge-amended soils were increased several times compared to those grown on the uncontaminated soils. Plants grown on the sludge-treated soils also accumulated elevated levels of Cu and Pb; however, the increases were relatively small.

Although relationships between soil properties and metal concentrations in edible parts of the food crops varied between metals and food crops, but metal accumulation in the plants were generally influenced by soil metal concentration, pH, $CaCO_3$ content and soil texture. A number of soil tests (EDTA, DTPA, $CaCl_2$, NH_4NO_3 and total metal concentration) were used to predict the accumulation of trace metals in the food crops. The results indicated that soil metal concentrations extracted with the EDTA, DTPA, $CaCl_2$ and NH_4NO_3 generally correlated well with metal concentrations in the edible parts of the plants. However, these tests tended to be crop or metal specific. In general, the total metal concentrations in the soils proved to be the most reliable predictor of metal concentrations in the food plants. The importance of trace metal concentrations in soils and soil properties in controlling the transfer of trace metals from sludge-treated soils to the food crops are discussed.

TRACE ELEMENT UPTAKE BY VEGETABLE CROPS FROM ALKALINE STABILIZED SEWAGE SLUDGE (N-VIRO SOIL) COMPARED TO DIGESTED SLUDGE

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A field experiment was established in 1993 in which an alkaline stabilized sewage sludge (N-Viro Soil) from Columbus, Ohio was applied to agricultural land at a rate of 500 mt/ha to simulate long-term agronomic applications, or one-time applications for reclamation or soil conditioning. Six vegetable crops (lettuce, cabbage, tomato, dry beans, carrot and potato) were grown on the treated plots and fertilizer controls in 1993 and 1994, and plant uptake slopes calculated for selected metals (Cd, Cu, Ni, Pb and Zn). Results were compared to those for City of Columbus anaerobically digested sludge applied in an adjacent area in 1989 at rates ranging from 100 to 2000 mt/ha. A common set of fertilizer control plots were used to calculate uptake slopes for both sludges. N-Viro Soil raised soil pH to 7.5 from the initial value of 6.5. Digested sludge initially increased pH above 7, but pH has declined with sludge rate from 1989 to 1994 to < 6.2 as a result of sludge N nitrification. Plant uptake of Cd and Zn, and to a lesser extent Cu, increased with sludge application for most crops, while Ni and Pb uptake were not significantly changed. Lettuce showed the greatest metal accumulation among the six crops studied. In 1993, there were no significant differences in metal uptake from N-Viro Soil for any of the crops, although there was a trend for increased Cd and Zn uptake in lettuce and decreased Cd, and Zn uptake in lettuce. N-Viro Soil reduced Pb uptake in most crops. In 1994, there were no significant differences in metal uptake by any of the crops.

Crop yields generally increased with sludge application rate, except in the first year after application where phytotoxicity was evident in lettuce. In subsequent years, crop yields have generally increased with sludge application. N-Viro Soil significantly increased the yield of carrots in 1993 and 1994, and lettuce in 1994.

TRACE METALS IN FRENCH SOIL HORIZONS: II. MOBILITY AND BIOAVAILABILITY TO WHEAT

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This work is a part of the French research programme named ASPITET (Apports d'une Stratification Pédologique pour l'Interprétation des Teneurs en Eléments en Traces dans les sols, *i.e. Contribution of pedological stratification for interpreting trace metal contents in soil horizons*).

A good quality soil should not have a negative effect on crop yields, nor produce harvested materials of a composition that could be harmful to animals or humans (McGrath, 1989). However, concentrations of trace metals in plants and foods may reflect geochemical background in soil as well as different anthropogenic sources of pollutants. The contributions of these pathways are not relatively well understood, especially in France. Now, the composition of French soils is extremely diverse as a result of various climatic conditions and parent materials. Consequently, further investigations are needed both on non-polluted and contaminated soils for many trace elements. However, much evidence indicates that the chemical composition of plants mirrors, in general, the composition of soil solution, but not necessary the total trace element content in the soil. Moreover, plant themselves have effects on dose response curves. There must be more concern about potatoes and cereals because these products form a major part of the French diet. So, the objectives of this study were to investigate (i) the mobility of trace metals in several non-polluted French soils derived from various parent materials and (ii) the bioavailability of trace metals to wheat (*Triticum aestivum*).

Total content of seven trace metals (*i.e.* Cd, Co, Cr, Cu, Ni, Pb and Zn) and other physico-chemical properties were determined on 420 French soil horizons, related to 170 forest or field sites in rural areas of the North Part of France (Baize, 1995, this Conference). Then, preliminary investigations on metal mobility and bioavailability were carried out on a sub-population which included 16 sites, located in the Yonne district (France), and five soil types (FAO-UNESCO, 1989; Baize, 1992): *i.e.* Calcaric Cambisols from carixian marls ("Carixien"), Rendzic Leptosols over sinemurian limestones ("Terres noires", Stagnic Luvisols over sinemurian limestones ("Sols marrons"), Eutric Planosols from domerian clays ("Sols planosoliques du Domérien") and Chromic Luvisols (truncated) over jurassic limestones ("Terres d'Aubues rouges"). Metal mobility was evaluated using single step extractions by 0.1 N calcium nitrate, EDTA-ammonium acetate pH 7, and DTPA. Shoots (at stem elongation stage) and grains (at harvest) were collected in fields. Dried plant samples were milled in a zirconium oxide grinder. All resulting powders were mineralized by both dry ashing and wet (digestion) ashing. Metal concentrations in soil extracts and plant ashing solutions were measured by AAS, ZETAAS or ICP depending on concentration range.

Attention was focused on the relationships between metal concentration in plant samples and (i) metal mobility in soil, (ii) total metal content in soil and (iii) pedological parameters. In addition, metal removal by harvested grains were calculated.

For example, Zn total content in soils ranged from 88 mg to 1828 mg/kg DW, while Zn extracted by EDTA-ammonium acetate ranged from 2 to 90 mg/kg soil DW. Zn concentration in

wheat shoots varied from 15 mg to 60 mg/kg DM. Shoot-Zn concentrations were not randomized, and reflected the characteristics of the soil horizons under investigation. The highest Zn concentrations in shoots were found for wheat plants grown in the "Terres noires" soils and the lowest ones in the "Terres d'Aubues" soils.

In conclusion, this preliminary work would be an interdisciplinary and basic tool leading to further projects giving information on:

- natural occurrence of trace metals in French soils and plants, depending on parent material, soil type and horizon, and land use;
- reference and threshold values for detecting and assessing antropogenic contamination;
- tentative guideline values for mobile and plant available metals in French soil horizons.

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DISTRIBUTION OF ^{99}Tc IN WINTER WHEAT GROWN ON CONTAMINATED SOIL

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^{99}Tc is an artificial radioelement which is found in nuclear waste. This long-lived radionuclide is very mobile in soils when present in oxidized form. Plants generally take up readily ^{99}Tc and transfer the element in the aerial parts (Cataldo *et al.*, 1983; Vandecasteele *et al.*, 1983; Myttenaere *et al.*, 1986; Echevarria *et al.*, 1994). This work was undertaken to study the fate of ^{99}Tc into the shoots of a crop to assess the risk connected to contamination of agricultural soils by radionuclides and its consequence on Tc content of Man-consumed parts of the crops.

Winter wheat (*Triticum aestivum* L., cv Frandoc) was used as test crop. Seeds were humidified, and after germination, placed for 40 days at +4°C to allow for their vernalization. Soil samples were collected from the Ap horizon of a calcic cambisol. After air-drying and sieving at 5 mm, Tc was added to the soil at three rates (2.5, 25, and 250 kBq kg^{-1}) in the form of NH_4TcO_4 1.5 kg of contaminated soil was placed in plastic tubes (8 x 40 cm) and humected at the water holding capacity. One seedling was planted per tube and allowed for growing in a growth chamber with the following conditions: 16 hour photoperiod, 24°C day and 15°C night, 80% humidity. Soil humidity was maintained at the water holding capacity. The height of the shoots, the number of suckers and the date of earing were recorded. At grain maturity, the different parts of the plants were separated, dried at 70°C, weighed and their content in Tc was determined by liquid scintillation counting after acid mineralization.

Results showed that the presence of Tc in soil at a rate of 250 kBq kg^{-1} induced a reduction in wheat growth. This concentration in soil was 4 times higher than the phytotoxicity level reported earlier (Cataldo *et al.*, 1983). At the same rate of Tc contamination and on the same soil, growth of rye-grass had not been affected (Echevarria *et al.*, 1994). At high concentration in soil, Tc did not only affect wheat growth but also decreased the number of suckers per plant and caused a one-week delay in earing. At maturity of control plants, humidity of the grains in presence of 250 kBq kg-1 was significantly higher than for other treatments. Transfer factors (DPM g^{-1} dry plant material / DPM g^{-1} dry soil) were high in general except for the grain. They were higher in leaves ranging from 285 to 351 corresponding exactly to the values observed in rye-grass leaves on the same soil with the same concentrations of Tc (Echevarria *et al.*, 1994). Transfer factors in stems, husks and roots ranged from 8 to 28, and were lower than 1 for the grain. These results indicate that Tc is mostly stored in plant leaves and that roots and stems are only transit organs. Once it is stored in leaves, Tc may be remobilized towards the grain. Accumulation of Tc in shoots reached 41 to 49% of initial Tc. Again, most of the radionuclide was recovered in the leaves (more than 90%) while only less than 0.1% was found in the grain.

These results indicated that accumulation of Tc in cereals was high, but the radionuclide was found mostly in the leaves. Concentration of Tc in the grain (consumed part) was unsignificant compared with the initial rate of Tc in soil. However, high accumulation of Tc in leaves has to be taken into account as leaves may be recycled into the soil after the crop. In our case, when soil contamination was made with contaminated leaves, hydrosoluble Tc represented 70% of total supply of Tc. Incorporation of crop residues into the soil may therefore contribute to supply Tc to future crops in a rather mobil state.

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CADMIUM UPTAKE AND BIOACCUMULATION IN SELECTED CULTIVARS OF DURUM WHEAT AND FLAX PLANTS AS AFFECTED BY SOIL TYPE

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The potential for Cd contamination to the food chain through the soil-root interface has received increasing attention. Although Cd uptake varies considerably with plant species, the processes which determine the accumulation of Cd in plant tissues are affected by soil factors. The influence of soil type on Cd uptake by durum wheat (*Triticum turgidum* var. *durum* L.) and flax (*Linum usitatissimum* L.) was studied in a pot experiment under environmentally controlled growth chamber conditions. Four cultivars/lines of durum wheat (Kyle, Sceptre, DT 627, and DT 637) and three cultivars/lines of flax (Flanders, FP 935, and YSED 2) were grown in two types of Saskatchewan soils: Orthic Gray Luvisol (low background Cd concentration; total/ABDTPA extractable Cd: 0.12/0.03 mg/kg, respectively) and Dark Brown Chernozemic (relatively high background Cd concentration; total/ABDTPA Cd: 0.34/0.17 mg/kg, respectively). Plant roots, stems, newly developed heads, and grain/seeds were analyzed for Cd concentration at three stages of plant growth: two and seven weeks after germination, and at plant maturity. The results showed that Cd bioaccumulation and distribution within the plants were strongly affected by both soil type and plant cultivar/line. The Cd concentration in different parts of plants varied at different stages of plant growth. However, all cultivars of both plant species grown in the Chernozemic soil accumulated more Cd in grain/seeds than plants grown in the Orthic Gray Luvisol soil. This also corresponded to the levels of ABDTPA extractable and metal-organic complex bound soil Cd in both soil. Large differences were found in grain Cd among the durum wheat cultivars groan in the same soil type, suggesting the importance of rhizosphere processes in Cd bioaccumulation and/or Cd transport processes within the plant. Distribution of Cd in parts of mature plants showed that durum grain contained up to 17 and 29% of the total amount of Cd taken up by the plants for the Orthic Gray Luvisol and Chernozemic soils, respectively. These results indicate the importance of studying Cd speciation, bioaccumulation and cycling in the environment for the management of agricultural soils and crops.

SPECIATION OF FLUORIDE AND UPTAKE OF FLUORIDE BY PLANTS

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Introduction. Fluoride (F) is normally present in low concentrations in plant material, yet is a major pollutant emitted from aluminium smelters, fertiliser factories and brick works. Phosphatic fertilisaers contain up to 3.0% F. Much research has investigated the atmospheric effects of gaseous and particulate F on F phytotoxicity. However little work has been done on uptake of F by plant roots as a pathway for movement of F from polluted soils to plants. There is some disagreement on the species of F taken up by plants (1,2). Under acid conditions, F can exist as the free ion or in a number of complexes in solution with Al, H, Si and B. The aim of the experiments was to determine which species of F: (a) are taken up by oat plants, and (b) are phytotoxic to oat plants.

Materials and Methods. Oats (*Avena sativa* cv. Echidna) were grown in a solution culture (3) with 25 μM P. Treatments included: 0, 18.5, 37, 74, 185, 370, 741 μM Al; 0, 1684, 3368, 6736 μM F; the following ratios of Al:F, 185:1684, 370:1694, 556:1684 and 741:1684 μM; growth at pH 3.5, 4.0, 4.5, 5.0, 6.0, and 7.0 with and without 1684 μM F. Activities of ions in solution were calculated with GEOCHEM-PC. All experiments involving Al or Al-F were carried out at pH 4.2±0.1 and experiments involving F only were carried out at pH 6.7±0.2, unless otherwise specifies. The pH was checked daily and adjusted with 0.1 M HCl or NaOH when necessary. All treatments were replicated three fold. Eight oat seeds, germinated in deionised water, were planted into 8.7 L of solution culture when the radicle length was between 2.0 and 3.0 cm (day 1). All experiments were conducted in growth cabinets at 25±1°C with 12 h day and night periods. Light irradiance was 230±35 μmol s^{-1} m^{-2}. Treatments were introduced on day 4, additional micronutrients added on day 8, and the solution completely replaced on day 12. At no time were any nutrients limiting. Plants were harvested on day 15.

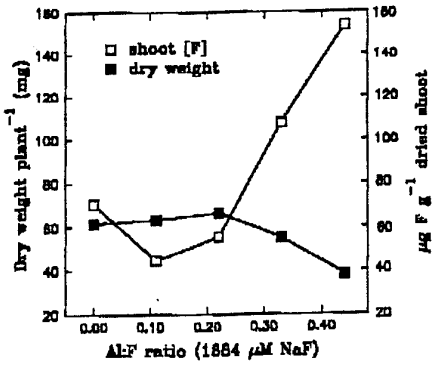

Figure 1. Concentration of F in oat shoots and plant dry weights, Al-F experiment.

Results and Discussion. Shoot and root dry weigths of oats were unaffected by any treatment where F was present as the free uncomplexes ion in solution, shoot F concentrations with

these treatments were as high as 940 μg F g^{-1} plant (data not shown). Dry weights of oat plants were significantly ($p<0.001$) decreased when Al_h (Al^{3+}, $AlOH_2^+$ and $AlOH^{2+}$) activities in the absence of F exceeded 37 μM. However, when Al and F were both present in solution, decreases in plant growth could not be explained by solution Alh activities alone. The presence of Al-F complexes in solution restricted plant growth at activities of Al_h (<0.1 μM) which were non-phytotoxic in the absence of F. As the Al:F ratio in the solution increased, AlF^{2+} and AlF_2^+ concentrations increased, AlF_3^0 and AlF_4^- first increased before declining, while F concentrations in the shoots initially decreased from the control before increasing (Figure 1).

When the F concentration in solution culture was constant (1684 μM), plant growth declined as pH decreased below 4.5, increasing HF activity in the solution. The decrease in growth was correlated with an increase in F concentration in the shoots (Figure 2). The growth decrease was significantly greater ($p<0.05$) with plants grown in the solution containing 1684 μM NaF (pH 3.5) when compared to plants grown in solution at pH 3.5 with no F. Fluoride and complexes with B and Si are currently being studied.

The data suggest: (a) that some species of Al-F are indeed phytotoxic and are readily taken up by plants, (b) that the F ion is more readily taken up by the plant than some species of Al-F, yet is less toxic, and (c) that HF is toxic and readily taken up by plants. Therefore, when assessing F uptake and phytotoxicity to plants, it is important to consider the concentration of F and the species of F available to plant root.

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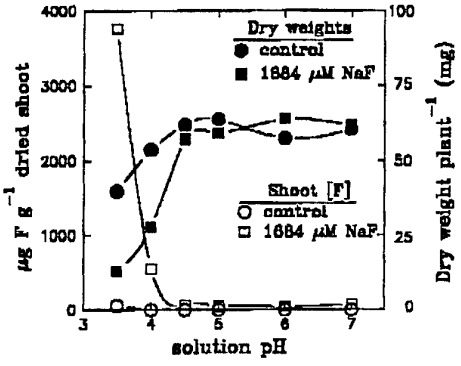

Figure 2. Concentration of F in oat shoots and plant dry weights, 1684 μM F and varied HF activity.

CADMIUM UPTAKE AND DISTRIBUTION IN THREE LETTUCE CULTIVARS

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Cadmium is a toxic heavy metal that can be introduced into the human food chain, by plant uptake from contaminated soils. This metal is very mobile in the soil/plant system, though plants present different capacities to uptake and translocate the element. Lettuce (*Lactuca sativa* L.) is known to accumulate cadmium in the above-ground tissues.

In the present work we compared the uptake and accumulation of cadmium by three cultivars of lettuce, Salinas, Atracção and Vanity. Plants were grown in nutrient solutions to determine their tolerance to cadmium. The cv Vanity was more tolerant than the cv Salinas, for which 20 mg L^{-1} Cd proved to be a lethal dose.

Plants were also grown in a sandy soil enriched with cadmium. Levels assayed were 0, 1, 3, 10, and 20 Cd kg^{-1} of soil. After one month of growth, plants were collected and separated into four parts: roots, stems, "old" leaves and "young" leaves. Results of the different distribution of cadmium in the three cultivars will be presented.

EFFECTS OF SOIL CONDITIONS ON THE BIOAVAILABILITY OF CADMIUM AND LEAD IN SLUDGED AND INORGANICALLY CONTAMINATED SOILS

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Cadmium and lead are both important elements with regard to their toxicological impact on human health. Sewage sludges are ubiquitous and important sources of several heavy metals, including cadmium, lead, zinc, copper. With regard to considerations of bioavailability, sludges are particularly important because they contribute significant amounts of organic matter to soils as well as plant macronutrients and heavy metals. This organic matter has several important effects on the cycling of metals; it acts as a sorbent which binds metals and also as a potential source of acidity as this organic matter is mineralised. Among the inorganic sources of heavy metals, mining and smelting are very important because they result in widespread contamination of soils and waters with a range of metals which are often in the forms of particles of sulphide ores. These have an acidifying action as they become oxidised in the soil. Although located in discrete geological regions, metalliferous mines can have important impacts on human health through the food chain. Land adjacent to mines is contaminated by fine particles of wind-blown tailings and spillages of contaminated processing waters. The tailings may continue to have a major contaminating effect long after the closure of the mine. Many historic mines have tailings heaps which have continued to be eroded and transported into the environment for more than 100 years. An important aspect of this source of metal contamination is the variation in the minerals associated with the ores (gangue minerals) and the mineralogy of the soil parent in the soils affected. Some ores contain calcite, which has a major neutralising and metal-retarding effect, while other ores do not. Silica and/or pyrite gangue minerals may also influence the behaviour of metals. Other regionally important sources of inorganic metals contamination include, fallout from metal smelters and metal processing industries (solid and liquids).

The results to be presented would be drawn from studies of soils from sludged and other contaminated field sites in the UK and from greenhouse and laboratory experiments with sludge-soil mixtures and metal salt-spiked soils over several years. This large supply of data enables several important conclusions to be reached with regard to the dynamics of metals in soil-plant systems and the paper will concentrate on the importance of pH, organic matter content of the soil, the carbonate content of the soil the redox status of the soils and trends in the changes of these properties over time.

Soil pH is of major importance in the availability of metals to plants, it will be shown that liming a range of sludged soils to pH 7.0 effected a reduction of up to 43% in the cadmium concentrations in lettuces and cabbages. The highest concentrations of lead and cadmium were found in the most acid soils of comparable total metal contents. In general, metals were found to be less available from sludged soils than from organically contaminated soils and this is probably due mainly to the higher organic matter content of the sludged soils.

Spiking soils to comparable total concentrations as contaminated field soils resulted in very much higher transfer coefficients of the metals. In general, soils spiked with salts showed 3 to 5 times greater metal uptake by ryegrass grown under identical conditions to the same crop on sludge soil mixtures with the same total contents. While redox conditions can affect the speciation of

metals, their effect on the contents of hydrous iron and manganese oxides which sorb metals is also very important in the dynamics of metals.

Trends over time in soil organic matter and pH in sludge soil mixtures will be shown.

A SURVEY OF THE CADMIUM CONTENT OF BRITISH WHEAT GRAIN

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The concentration of Cd in agricultural produce can have serious implications for its sale in the international community. This is because Cd is non-essential and one of the most toxic metals to animals and humans.

Generally, there is a lack of information on the Cd status of wheat grain from relatively 'unpolluted' agricultural land. The aim of the present study was to determine the Cd content of 400 and 393 samples of whole wheat grain collected in 1992 and 1993, respectively, from the major cereal growing areas in Britain. In addition, grain Cd was determined in 242 samples from a similar survey in 1982 which had been archived at Rothamsted. About 6000 samples of top soil were taken from England and Wales and analyzed for total Cd (McGrath and Loveland, 1992). The grain Cd content was compared with the total Cd content of British soils to see if a general relationship exists.

The ranges and median Cd concentrations ($\mu g\ g^{-1}$ dry weight) in wheat grain for the 1982, 1992 and 1993 surveys were 0.016-0.18, median 0.045; 0.008-0.27, median 0.035; and 0.004-0.31, median 0.034 respectively. There was about a 20% reduction in mean grain Cd content from 1982 to 1992/93. Differences between wheat varieties were small, and significant ($P<0.01$) only for 1992. When samples were separated into three groups representing breadmaking wheat, soft and hard wheat for fecd, there were no significant differences between groups. The percentage of samples above the World Health Organisation's (WHO) wheat grain limit of 0.12 μg Cd g^{-1} DW were 4% in 1982 and 1992 and 2% in 1993. The geographical distribution of these 'high' Cd grain samples corresponded with areas where the total soil Cd concentrations were significantly above background concentrations.

Bran Cd was significantly correlated with white flour Cd ($R^2 = 0.75$). On average, bran Cd concentration was 3 times higher than that of white flour.

The estimated daily dietary Cd intake in the UK through the consumption of wheat based products was about 8 μg Cd per person or about 11% of the WHO's limit of 70 μg Cd day^{-1}. It is concluded tha the Cd content of the vast majority of British wheat grain would not constitute a health hazard to the population of the UK or elsewhere, as it is well below the WHO's limit of 0.12 μg Cd g^{-1} DW.

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METAL AND METALLOID TRANSFER FROM SOILS TO MAN THROUGH VEGETABLES CULTIVATED IN POLLUTED SOILS: RISK ASSESSMENT AND EFFICIENCY OF METAL IMMOBILIZATION

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Local soil accumulations of zinc, cadmium, lead and arsenic are found in the vicinity of old non-ferrous smelters and of specific arsenic refineries. The large scale utilisation of arsenic in pesticides was a further important source for enrichment of this element in the environment.

Speciation plays an important role in the assimilation and toxicity of arsenic by living organisms; the trivalent state is more toxic than the pentavalent form.

In this study, the transfer of zinc, cadmium, lead and arsenic from polluted sandy soils to man through vegetables of several species was studied in kitchen-gardens in the vicinity of 2 old industrial sites in Belgium: (a) a former zinc smelter where the pyrometallurgical process was used for more than 70 years and (b) a former arsenic refinery. In the first case, the garden soils are predominantly enriched with cadmium (3 - 10 mg Cd/kg), zinc (90 - 1000 mg Zn/kg) and lead (170 - 470 mg Pb/kg). At the second site, the soil is contaminated with arsenic (40 - >200 mg As/kg soil); the concentration of other elements (Cd, Zn, Pb) is slightly increased but remains below the Belgian legal threshold values.

Accumulation of these elements was studied in the consumable parts of various species of vegetables (e.g. bean, spinach, lettuce, radish, carrot, potato) cultivated in several polluted gardens and compared to the content in the same species (and cultivars) grown in a non-polluted control garden with a comparable sandy soil. On the basis of a mean menu, weekly intakes of cadmium, zinc, lead and arsenic were calculated.

In former studies, it was shown that beringite (a modified alumino-silicate), hydrous manganese oxide and steel shots markedly decrease plant availability of Zn, Cd and Pb. The efficiency of beringite was tested *in situ*. Marked reduction of metal uptakes by the vegetables was observed. On the arsenic polluted soils remediation tests were performed with several products, which were expected to immobilize arsenic. Product selection was based on arsenic speciation. Steel shots were shown to possess a high arsenic immobilizing capacity. On arsenic polluted soils from the field treated with this product, arsenic assimilation by plants grown in a greenhouse was strongly reduced. Field experiments are in progress.

HEAVY METAL CONTAMINATION IN SOIL, WATER AND EDIBLE VEGETABLES AROUND A SMELTING FACILITY

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The study investigates the heavy metal contamination of soil, vegetation and surface water from a lead smelter and identify the pathways into the food chain. Samples of soil, edible vegetables and water were collected at varying distances from a smelting plant in a major mining region of Nigeria. The analyses of heavy metal contents (Pb, Mn, Cu, Zn, Sn, Sb, Cd, Zr, Ti) of the samples were obtained from a combination of X-ray fluorescence (XRF) and atomic absorption spectroscopy (AAS). Heavy metal burden in the soil, water and vegetables were highly elevated as indicated by soil toxicity potential (0.3 - 26) and index of soil surface contamination (2 - 14). The maximum allowable concentrations of these toxic metals are exceeded many times in soils for distances up to a few kilometers from the smelter. The concentrations of the toxic metals in the stream adjacent to the smelter is about three orders of magnitude higher than their content in unpolluted streams in the country. The high values of soil to plant transfer ratios ($\approx$12 for Pb) show that the toxic metals are largely transferred from polluted soils and water through vegetables into the food chain leading to injury to man and livestock. The calculated total metal intake from vegetables grown on the contaminated soils is about 2 to 3 orders above FAO/WHO prescribed levels. Recommendations on adequate solid/liquid waste management practices required for remediation are proposed.

EFFECTS OF SELENIUM SUPPLEMENTATION ON CHRONIC ACTIVE HEPATITIS

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Low serum selenium (Se) concentration may be associated with malignancies, cardio-vascular and liver disease. The biological role of selenium is related to glutathione peroxidase (GSH-PX), an Se-containing enzyme which protects cellular membranes against lipid peroxidation. Experimental studies have demonstrated that selenium deficiency leads to liver cell necrosis, which can be prevented by selenium supplementation.

In the present study we investigated the changes of serum selenium concentration and GSH-PX activities in whole blood with chronic active hepatitis, liver cirrhosis, hepatocellular carcinoma and the effects of selenium supplementation on chronic active hepatitis.

We included in the study 150 patients (118 males, 32 females) admitted to hospital for chronic active hepatitis, liver cirrhosis and hepatocellular carcinoma; the mean age was 52.3 years and range 21-75 years. 45 patients (35 males, 10 females) with chronic hepatitis received selenium supplementation for 12 weeks. The supplements were given four tablets of Se-rich yeast (XiLiKang) and provided 200 μg of selenium a day per subject.

Selenium concentration was measured by the fluorimetric method of Koh and Benson (1983); GSH-PX activities assayed by the method of Pleban *et al.* (1983).

The results showed that the serum concentration of selenium and GSH-PX activities in patients with chronic active hepatitis, liver cirrhosis and hepatocellular carcinoma were significantly lower than those in normal control ($p<0.01$). Serum selenium level was related to the severity of liver disease.

The reduction of ALT levels and improvement of hepatic histologic damage were observed after selenium supplementation. Selenium concentration and GSH-PX activities increased significantly ($p<0.01$), as compared with controls.

It suggested that the deficiency of selenium might be closely correlated to liver cell damage and development of hepatocellular carcinoma. Selenium supplementation may protect against oxidative liver damage, thus modifying the development of chronic liver disease.

BIOAVAILABILITY OF TRACE ELEMENTS FROM SEWAGE SLUDGE IN SOIL-PLANT SYSTEM

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Farming intensification requires to look for new organic fertilizer sours. A great deal of sewage sludge which is accumulated in waste treatment plants (from 0.5 to 1% of total volume of discharge) is a significant reserve of nutrient production. Sewage sludge recovery is a topical problem in terms of economy and environmental protection.

In the North-West Region of Russia, particularly in the Arkhangelsk Region with low-humic arable soils, the application of waste of pulp-and-paper mills such as sewage sludge, bark, lignin and composts on their base is very promising. Agricultural recovery of pulp-and-paper mills waste proses problems of investigations of environmental variations which happen in the agrocenosis.

Our investigations were carried out in the Arkhangelsk Region as a part of full-scale experiments of the Arkhangelsk Reclamation Pilot Plant. Effects of two rates (30 t/ha and 60 t/ha) of sewage sludge of different ages (1 year and 3 years) in combination with phosphate-potassium fertilizers on agrochemical properties of arable sod-podzolic soil as well as on contents of mobile forms of most toxic heavy metals were studied. Heavy metal delivery into plants was studied. Chemical composition of sewage sludge and composts based on it which were used in our experiments are listed in Table. In 1991, a vegetative experiment was begun simultaneously with our field experiment.

Waste type	pH	Moisture	Ash	Ntot	P205	K20	Ni	Zn	Pb	Cr
				%				mg/kg		
# Ss, 1-year	7.5	70.2	49.2	2.0	1.30	0.17	70.6	163.9	7.62	74.6
Ss, 3-years	7.1	69.8	57.1	2.2	1.10	0.11	180.1	180.6	10.10	112.7
Bark	5.3	72.0	91.0	3.50	1.50	1.20	2.78	93.88	0.10	4.5
Lignin	3.2	13.0	88.0	3.20	0.80	0.04	1.65	39.45	0.16	8.4
Sludge-bark	7.5	69.7	77.7	1.73	0.09	0.70	78.41	102.00	3.42	67.0
Sludge-lign.	7.4	69.0	75.0	1.25	1.00	0.11	57.60	115.20	5.76	55.0

#Ss - sewage sludge

Results of our analysis of the field experiment soil indicate that sewage sludge significantly affects all types of soil acidity and saturation of soil with bases. Reduction of the soil acidity and saturation of the soil absorbent complex with Ca and Mg decrease the mobility of heavy metals in the soil that in turn influences the delivery of heavy metals into plants. Sewage sludge age did not significantly affect acidic-and-basic properties of the soil. Three-years sewage sludge was characterized by higher concentrations of heavy metals because of sewage sludge transformation during the storage. Such feature of the 3-years sewage sludge had an effect on a content of mobile forms of heavy metals in the soil.

Phosphate-potassium fertilizers (simples superphosphate, potassium chloride) applied together with sewage sludge increased the content of mobile forms of all heavy metals under study. Probably, this is connected with acidifying action of both simple superphosphate and potassium chloride. During vegetative experiment when double superphosphate was applied, such effects of mineral fertilizers on the mobility of heavy metals in the soil were not observed. The content of mobile compounds of lead and nickel in the soil after one application of the sewage sludge did not

vary and reflected a natural variability. The content of zinc and chromium was reliably increased when sewage sludge and its combinations with mineral fertilizers were applied. Contents of Zn and Cr in the soil did not reach maximum allowable concentrations.

Chemical analysis of waste indicates that the sewage sludge can be a good organic fertilizer with reclaming action, bark and lignin can be used as an organic fertilizer after the composting. Sewage sludge application as organic fertilizer should be accompanied by the monitoring of the content of heavy metals in the soil and plants.

THE DISTRIBUTION OF GERMANIUM (Ge) IN THE ENVIRONMENT AND GINSEN RADIX ETC. CHINESE TRADITIONAL HERBS

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Germanium is an element lies thirty two position on periodic table of element, it is closed nealy arsenic and has similar chemical properties of arsenic, Ge is rare element in the earth.

A high concentration of germanium in Ginsen radix (Panax Ginsen), Gyrophora (Ganoderma Lucidum) Ructas Lycii (Lycium Chinese Mill) Bulous Allii (Allium setivum) etc. Chinese traditional herbs were described only by in 1971, in Japan. However this only one description of high Ge concentration has been quoted and exaggerated wide-spreadly by some Chinese scholars and many germanium-merchants in 1985-1993 in China, and give rise to some serious results, until we are correct this recognition. But, many studies and determinations have been confirmed that the concentrations of Ge in rocks, soils, river waters, mineral waters, foods, Chinese traditional herbs (including Ginsen radix which growthing and producting in China, Japan, Korea etc), plants and animals tissues were found from less than 1 ppm to 10 ppm. It rarely occurs in high concentration in nature and biological organism.

As for "Ginsen radix, Ganoderma Lucidum, Allium setivum etc. Chinese traditional herbs containing of large amount of organic germanium or carboxyetnylgermanium sesquioxide (Ge-132)", "Ge-132 is common importance effective composition of a lot of Chinese traditional herbs". "Organic germanium has not any side-effects and toxicity", "Ge is essential trace element of human beings" etc., all are false and exaggerated propaganda and advertisements. And have been corrected these series mistakes by us through two Germanium Research and Application Conferences of whole China and published many researches and monographies in 1993-1994.

SYMPOSIUM B4

EFFECTS OF PHOSPHATE ADDITION ON BIOAVAILABILITY OF LEAD IN CONTAMINATED SOIL FED TO RATS AND PIGS

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We have shown previously that addition of hydroxyapatite (HA) or phosphate rock (PR) can reduce the water solubility of Pb in Pb-contaminated soil by the precipitation of highly insoluble Pb phosphates, the pyromorphites. Before phosphate addition can be promoted as a viable *in situ* treatment approach for Pb-contaminated soils, it is important to determine if this treatment will reduce soil-Pb bioavailability from direct ingestion human exposure. In this study, Pb-contaminated soil with and without phosphate addition, and with appropriate experimental controls, were fed to rats. Pigs were also fed to evaluate a limited number of the treatments.

A soil containing approximately 8000 mg Pb/kg soil (dry solids), obtained from a former Pb mining area in Butte, Montana, was used for the feeding study, which was conducted at Louisiana State University. This soil is currently undergoing extensive chemical and mineralogical characterization by Ohio State University and USEPA and the results will be used to support findings of the feeding trial. Rats were fed soil at 2.5% of their diet by weight, so as to give a total Pb concentration in the diet of 200 mg/kg. All other treatments were added to the diet so as to keep the Pb concentration constant. Lead acetate was used as 100% bioavailable Pb source, uncontaminated mineral soil (US) was obtained from the Ohio State University farm in Columbus, hydroxypyromorphite (HP) was synthesized by precipitation from aqueous solution, reagent grade hydroxyapatite (HA) was obtained from Bio Rad Co., and Florida phosphate rock (PR) was obtained from the Occidental Co. The treatments included:

Animal feed control (AFC)
AFC + Pb acetate
AFC + hydroxypyromorphite (HP
AFC + uncontaminated soil (US)
AFC + US + Pb acetate
AFC + US + HP
AFC + US + Pb acetate + hydroxyapatite (HA)
AFC + Pb-contaminated soil
AFC + Pb-contaminated soil + HA
AFC + Pb-contaminated soil + phosphate rock (PR)

A cohort of five rats represented each treatment. Rats were fed for five weeks, euthanized, and blood and femur samples taken for Pb analysis. Samples of the diet and feces were also taken for analysis. Samples were shipped from LSU to OSU. Blood samples were digested in ultrapure conc. HNO_3, and analyzed by graphite furnace AA. Femur samples were ashed at 500°C and then digested in conc. HNO_3-$HC1O_4$.

Two cohorts of two pigs each were fed AFC + US + Pb acetate and AFC + US + HP. Samples of blood and bone were analyzed as described for rats.

Initial sample analysis indicates that Pb bioavailability is directly related to solid phase Pb speciation. Up to 100-fold differences in blood Pb were found among the treatments. Detailed discussion of the results will be presented.

CHRONIC LEAD TRANSFER FROM POLLUTED SOILS TO MEN BY MEANS OF INHALATION AND INGESTION

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Since low-level, long-term exposure is the most common way of exposure to environmental metals, the possible chronic effects of these agents are of great concem to toxicologists, regulatory officials and the general public.

In an industrial situation there may be a clear history of exposure even if there is just the domestic environment to be judged.

The first step to elucidate possible health effects due to chronic lead incorporation from contaminated soils and food of vegetable origin grown on those soils consists in the evaluation of lead in human body fluids or teeth thus trying to find a correspondence to the contamination of soil and of food of vegetable origin from their surrounding environment.

We present the results of an investigation carried out at the surrounding soils of an industrial site where lead has been processed for decades and where in addition families have grown vegetables and fruits for their own consume in home gardens for years.

The soil of home gardens revealed a severe contamination due to lead, with maximum amounts of 5.660 mg lead per kg of soil, while the general soil contamination at the industrial site mounted up to nearly 10.000 mg lead per kg soil.

41% from the home grown food of vegetable origin presented lead contents beyond the fixed lead tolerances appliable to food, with some specimen showing remarkable high values.

Blood lead levels of 1.41 1 inhabitants of the lead contaminated zone were determined by AAS showing that the lead concentration in blood depended mainly on the distance of homes from the industrial site while the intake of food of vegetable origin from home gardens increased the blood lead level by up to 50%, with children showing the highest mean value rates. 18,3% of explored children showed increased blood lead levels (> 15 µg/ 100mL).

This effect of lead accumulation in the food chain directed the way to measures by the environmental and public health authority (e.g. lead emission reduction, home garden earth exchange, public and private hygiene awareness) to reduce the possible effect of chronic lead exposure on human health.

Two and a half years after these measures a new medical examination with analysis of blood lead levels took place.

EXPOSURE OF YOUNG CHILDREN TO METALS THROUGH HAND-TO-MOUTH ACTIVITY

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It has been estimated that the 2-year old child involuntarily ingests around 100 mg of soil/dust per day, though studies using stable markers such as Ti, Al and Si indicate that this may be too high. Measurements of exposure have shown that hand-to-mouth activity accounts for approximately 50% of the total lead intake by urban children of this age.

A number of studies undertaken at Imperial College have demonstrated extensive soil contamination with metals in areas of past mining and smelting activities in Britain, with high concentrations of potentially toxic elements in both household garden soils and indoor dusts. Significant amounts of metals have been found on children's hands.

The paper presents two case histories based on studies undertaken in (a) the Pb/Zn mining area in the Southern Pennines in which some 250 km^2 of urban and rural land contain several hundred to several thousand μg^{-1} Pb and (b) an arear of Cu-Sn-As mining in south-west England in which 700 km^2 of land is contaminated with arsenic ranging up to 2,500 $\mu g\ g^{-1}$ in soils and up to 460 $\mu g\ g^{-1}$ in household dusts. In the former area, the geometric mean loading of lead on the hands of children aged 1-2.5 yrs was 13.1 µg Pb per pair of hands, significantly greater than the mean of 5.7 µg Pb previously reported in the inner-city area of Birmingham. Microscopy based tracer studies have clearly demonstrated the movement of particulate lead from soil (sometimes via housedust) onto the hands. Lead isotope studies have confirmed that some of this lead is ingested and absorbed. However high lead intake was only to a small extent reflected in raised blood lead values, which had a geometric mean of 9.4 $\mu g\ dL^{-1}$. It is proposed that this is explained by low bioavailability due to the presence of a stable soil-lead mineral, pyromophite, which has an extremely low solubility. This mineral was found to be a major lead-bearing constituent in the soils, dusts and wipes from children's hands.

In old mining villages in Cornwall, direct ingestion of arsenic in soil/dust is thought to be as much as 30-40 µg As per day in the young child and to be the most important route of exposure in local communities. In this area, arsenic was mined as the mineral arsenopyrite and emissions of arsenic oxide were dispersed onto soils in the course of smelting operations. Present day speciation and bioavailability of arsenic in surface soils and dusts is unknown and presents an urgent research requirement.

ADVERSE HEALTH EFFECTS DUE TO SOIL AND WATER ACIDIFICATION A SWEDISH RESEARCH PROGRAM

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Environmental acidification is a widespread global problem, especially in areas with a combination of high fall-out of acid precipitation and low buffering capacity of water and soil. In light of the considerable effects in soil and water caused by acid precipitation, it is important to identify and quantify the possible adverse effects on human health. The Swedish Environmental Protection Agency is running a five year research program, started in 1991, on effects of acid precipitation on human health. The program is focused on indirect effects of acid precipitation, due to effects on the mobility of trace elements, giving in most cases an increase, but sometimes a decrease in metal levels. Through the food chain, the intake of toxic as well as essential elements may be altered in man.

The objective of the program is to elucidate 1) to what extent acidification will change human exposure to elements, either the total levels or the chemical form and 2) what this means to human health. The aim to find any effects as early as possible, in order to be able to prevent harmful effects in humans.

Altogether 15 research projects have been supported by grants from the program. The main activities have been focused on methods for exposure monitoring, both as measurements of intake via food and drinking water as well as determination of the absorbed dose, measured in human samples as blood, urine, breast milk and hair. Also epidemiological studies on the correlation between exposure of metals, related to acid precipitation, and adverse health effects have been studied in the program. Examples of results from the program, with significance for the risk assessment of metals and acid precipitation are described below.

In an area affected by acidification there was significant associations between pH of the drinking water and element concentration. The correlation was negative for aluminium, cadmium, copper, and lead and positive for calcium and magnesium. However, farmers, consuming this water as well as large amounts of locally produced foods, did not show any significant changes in blood levels of these elements. Neither the level of cadmium in the kidney, measured *in vivo*, was affected by acid precipitation.

- The copper intake from drinking water in infants and children has been studied in two Swedish cities, where the copper levels in drinking water was about 1 mg/l before flushing. The incidence of non-infectious diarrhoeas and other gastrointestinal symptoms in relation to copper exposure has been investigated and the results will be presented in 1995.

- The bioavailability of cadmium in different foods has been studied. Women with low seum ferritin levels (indicator of low or depleted iron stores) had elevated cadmium levels in blood and urine. Animal studies showed a similar level of bioavailable cadmium in drinking water and in solid foods as crab and mushroom. The bioavailability of cadmium in drinking water was reduced in the presence of organic matter as humic and fulvic acids.

- The uptake of cadmium in wheat and potatoes was shown to mainly be dependent on the level of cadmium in the soil, and to a lesser degree to pH.

- The mechanisms for uptake of mercury in fish via the gills and the food has been investigated. pH and the level of chloride ions in the water are of importance for the uptake of mercury in fish.

Conclusion: Although there are several indications that the exposure to toxic elements, e.g. cadmium, methylmercury and lead, as well as the intake of essential elements, e.g. selenium, may be affected by acid precipitation, there is presently no firm evidence of adverse health effects in man. However, the present data clearly indicate that the safety margins are small and effort should be made to keep all sources of exposure to toxic elements as low as possible. Thus the ongoing acidification in many areas should be stopped before adverse health effects become evident.

SELENIUN IN SOIL AND ITS EFFECT ON HEALTH IN CHINA

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Selenium is an essential element for human, animal and some microorganism species. In our recent studies we found that selenium exists in the glutathione peroxidase in higher plant (wheat, maize, rape, soybean and garlic) and plays the role of antioxidation through the enzymatic mechanism of glutathione peroxidase which is similar to that in animal and human bodies. Selenium can also stimulate the seedling growth of the above plant species. Therefore it is extremely possible that selenium could be an essential to higher plant, which has never been demonstrated in the world. The biological functions of selenium show a dual charater. Selenium content range between toxic and deficient level is very narrow. Selenium distributes unevenly in the surface of the earth and shows different selenium contents in different geoecosystem, forming seleniferous and Se-defficient geo-ecosystems to, in turn, affect health of human and animal, as well as the production development. Selenium contents in soils and its existing forms are important factors affecting its cycling, flux, and balance in geo-ecosystems.

This paper mainly deals with the geographical distribution of two forms (total and water-soluble) of selenium in top soils, as well its effect evaluation on health in China. Totally 352 top soil samples, including natural 157 and cultivated 195, were collected from the 21 main soil types in whole China. Selenium was determinated with DAN-fluoroscence spectrophotometry. Soil water soluble selenium was extracted with a water to soil ratio of 5:1.

It has been found that the average content of total selenium in all soils in the country is 0.239±0.320 mg/kg with a lowest of 0.022 mg/kg and highest of 3.806 mg/kg, among them for the cultivated soils the average of total Se is 0.265 0.381 mg/kg, higher than that in the natural soils, 0.206±0.227 mg/kg. It has been found that selenium contents vary in different soils. Based on the analysis on 21 main types of soils, the soils can be ordered as following according to their selenium contents (mg/kg): laterite (0.982±0.370) > yellow earth (0.549±0.532) > red earth (0.345±0.189) > dryland cultivated soil (0.287±0.421) > Chernozem (0.253±0.145) > black soil(0.235) > paddy soil (0.201±0.089) > solonchak (0.198±0.126) > grey forest soil (0.195±0.054) > desert soil (0.186±0.091) > chestnut soil (0.167±0.080) > calcic brown soil (0.154±0.072) > meadow soil (0.145±0.069) > brown earth (0.133±0.099) > swamp soil (0.122±0.032) > loessial soil (0.118±0.069) > red drab soil (0.111±0.056) > yellow-brown soil (0.101±0.050) > drab soil (0.098±0.032) > dark brown soil (0.096±0.025) > purple soil (0.086±0.048).

The average value of water-soluble Se in all soil samples is 6.4 μg/kg with a lowest of 0.6 μg/kg and highest of 109 μg/kg, the sequence based on water-soluble Se content (μg/kg): desert soil (18.3) > solonchak (13.1) > laterite (11.0) > chernozem (8.9) > yellow soil (7.1) > calcic brown soil (6.9) > dryland cultivated soil (6.5) > meadow soil (5.4) > chestnut soil (5.0) > red soil (4.2) > paddy soil (3.9) > grey-forest soil (3.7) > black soil (3.2) > swamp soil (2.4) > purple soil (2.2) > dark brown soil (2.0) > drab soil (1.9) > yellow brown soil and red brown earth (l.8) > brown earth (l.6).

From above we can see the two orders are different and this demonstrates that the proportions of the forms of selenium existing in soils are different. They can affect the Se-flux in geo-ecosystem and health of human and animals via food chain. So it is very important to

understand the proportion of the two forms of selenium in soil for risk evaluation of soil Se. We have calculated the percentage of water-soluble Se to the total Se and the soil orders show as following (%): Desert soil (9.88) > solonchak (6.63) > calcic brown soil (4.50) > meadow soil (3.71) >chernozem (3.52) > chestnut soil (3.52) > purple soil (2.56) > dryland cultivated soil (2.45) > dark brown soil (2.09) > swamp soil and drab soil (1.96) > paddy soil (1.94) > grey forest soil (1.90) > yellow-brown soil (1.79) > red drab soil (1.62) > black soil (1.36) > brown soil (1.33) > yellow soil (1.29) > red soil (1.22) > laterite (1.12).

We can see from the above order that the percentages of water-soluble Se to the total Se decrease from the soils developed in the northwest dry desert condition to those developed under the southeast humid-heat geographic condition: generally speaking, dry desert/steppe soil series shows the highest, while the tropic/subtropic forest soil series such as laterite, red and yellow earth the lowest, but their absolute water-soluble Se contents are still relatively high because of their higher total Se contents; and the temperate forest soil series is situated in the middle. The correlationship analyses between the total Se and water-soluble Se in soils show that there is a very significant correlation in cultivated soils with a coefficient of 0.85 ($p<0.001$), while in paddy soil and total natural soils the coefficients are 0.18 and 0.14 respectively, showing no significent correlation. However, for different type of natural soils there could be different correlativities depending on the soil types. Generally there are significant or very significant correlativities in meadow, desert and saline soils, while in forest soils they show no significent correlation.

Also this paper studied the relationship between selenium in soils and the human endemic diseases and selenosis, respectively resulting from deficiency and excess of selenium in soil and their influence on Se flux of food chain. It has been found and demonstrated in China that the incidences of Keshan disease and Kaschin-beck disease have been closely related with selenium deficiency in the geo-ecosystem, also human selenosis has occurred in the limited areas with seleniferous soil. The research result shows that the selenium content in soils of Keshan disease areas and Kaschin-beck disease areas are lower than those of diseases free areas. In the cultivated soil total selenium averages of endemic areas and non-endemic areas are 0.142 mg/kg and 0.292 mg/kg, respectively, and water-soluble Se averages in soil of endemic areas and non-endemic areas are 2.5 μg/kg and 6.0 μg/kg, respectively. As for natural soil, we have found that Keshan disease and Kaschin-beck disease are similarly distributed in a long belt ranging from Northeast to Southwest in middle China, and on its both sides (southeast side and northwest side) there exists two disease free belts; the natural soils with lower Se content, such as brown earth, dark brown earth, drab soil, purple soil etc... are mostly located within the disease belt. On the basis of soil selenium, the ecocycle and flux of selenium in geo-ecosystem within the disease affected and non-affected areas was discussed. In the end, the reference criteria for evaluating Se deficiency and Se excess in soil were determined respectively.

HEALTH RISK ASSESSMENT OF MANGANESE IN RELATION TO MMT IN GASOLINE

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The potential for manganese (Ma) exposure and health effects in relation to the use of methylcyclopentadienyl manganese tricarbonyl (MMT) in unleaded gasoline was evaluated by the U.S. Environmental Protection Agency (EPA). Although EPA primarily focused on the inhalation aspect of Mn exposure, other routes were considered. Based on epidemiologic findings of neurobehavioral effects in Mn-exposed workers, EPA derived an inhalation reference concentration (RfC) of 0.05 μg Mn/m^3. The RfC is defined as an estimate (with uncertainty spanning about an order of magnitude) of a continuous inhalation exposure level for the human population (including sensitive subpopulations) that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. Additional analyses involving dose-response modeling methods resulted in a range of alternative RfC estimates of 0.09 to 0.2 μg Mn/m^3. An exposure assessment relied upon the EPA Particle Total Exposure Assessment Methodology (Particle TEAM) study and other sources of information on Mn levels in the Riverside, California area, where MMT was used in leaded gasoline. The Particle TEAM study monitored outdoor, indoor, and personal exposure levels of particulate matter in a statistically representative sample of Riverside residents over a 24-hour period during the fall of 1989. By determining the automotive contribution to respirable Mn levels and extrapolating from the limited use of MMT in Riverside in 1989 to a scenario of 100% usage of MMT in unleaded gasoline, it was possible to predict a distribution of personal exposure levels of Mn. The characterization of public health risk incorporated a comparison of predicted Mn exposure levels with Mn RfC estimates. The methods employed in this assessment may provide useful guidance for other environmental health risk assessments.

HEAVY METALS IN URBAN SOILS OF SANTIAGO, CHILE. INTERRELATION TO ATMOSPHERIC POLLUTION

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The main problem of all researchers that study atmospheric, water, soil or other type of pollutants is the assignment of the sources of these pollutants. In many cases the origin of the pollutants is difficult to find due to the permanent relationship between all the components of the environment.

The present work corresponds to the study of urban and suburban soils and its relation to the suspended particulate matter in the city of Santiago. To evaluate the impact of the growing of the city (increasing of the industrial development and related aspects) the study includes the results in 1983 (as baseline) and those corresponding to ten years after.

The total suspended particulate matter (< 45 μm) were studied by forced aspiration over cellulose and glass fiber filters.

Samples of aerosols were taken simultaneously for 24 hours, at different urban and suburban sites of the city. Gravimetric and chemical analysis were done on the collected material.

Simultaneously, "local soils" corresponding to samples of soils around the aerosols's sampling sites were taken and characterized by physical and chemical analysis.

Samples of aerosols and soils (0 - 5 cm) were dissolved by wet digestion using a microwave digestor. The chemical elements were quantified using Atomic Absorption Spectrophotometry and Inductive Coupled Argon Plasma Spectroscopy.

The assignment of the origin of the elements was undertaken using the correlation coefficients and enrichment factors (FE) criteria.

Results show that Fe, and Mn have a natural origin in all samples, but other elements such as Pb, Cd, Cu, As, Se, and Ni are anthropogenic.

It was also observed that the assigment of the origin of an element can change when the interrelation soil-air is introduced. In Santiago (and certainly all along Chile), this situation is especially marked in the case of Cu.

In general, data obtained show that samples from the different sites are different, both for aerosols and for soils. Therefore, it is not possible to generalize results for the city with the data coming from only one site.

On the other hand, il is possible to conclude that the study of atmospherical pollution by heavy metals in the suspended particulate matter should be related to the study of "local soils" as possible sources of pollutants. However, the study of "local soils" should be reserved only to peripheric sites and not to urban sites such as down-town. In this type of sites a considerable recycling of pollutants between soil and air exists.

On the other hand, only for international comparisons a reference to the terrestrial crust could be useful.

INCIDENCE DE L'UTILISATION DES EAUX USEES EN AGRICULTURE : MODALITE DE LA CONTAMINATION D'UNE CHAÎNE TROPHIQUE TERRESTRE PAR LES METAUX TRACES

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L'étude de l'impact du plomb, du cadmium, du chrome, du cuivre et du zinc sur la chaîne alimentaire trophique (eau-sol-luzerne-bovins-homme) de la zone d'épandage a été abordée par une approche descriptive en milieu naturel, avec le souci permanant de confronter les résultats de tous les maillons analysés.

La principale source de métaux dans cette zone est l'eau usée qui est utilisée directement pour l'irrigation des sols. Le transfert des métaux à partir du sol vers les autres maillons est important, ce qui justifie l'hypothèse de la « biomagnification » de certains éléments étudiés comme le Cd.

Bien que n'ayant pas le même rôle dans le métabolisme, le cadmium (métal non-essentiel), et le zinc (métal essentiel) présentent des variatiom similaires dans la plante, tandis que le chrome, le plomb (non-essentiels) et le cuivre (essentiel) ont un comportement particulier. Par contre, des différences importantes ont été mises en évidence pour la bioaccumulation de ces deux groupes de métaux et leur régulation par les organes. Nous avons montré que dans le rein les interactions entre les divers métaux sont importantes surtout entre le Cd et le Zn.

Les concentrations en ces éléments dans les maillons testés sont importantes voir même très élevées dans les reins et le foie des bovins. Or même si ces concentrations ne sont pas nocives pour les animaux eux-mêmes, elles représentent des dangers potentiels pour les consommateurs ultérieurs, dont l'homme constitue un maillon final. Ainsi, des teneurs importantes de ces éléments ont été notées dans les cheveux de la population vivant dans cette zone. Ce qui reflète une forte pollution de cette population par voie trophique.

Mots clés : Eau usée, métaux lourds, chaîne alimentaire, Marrakech.

A STUDY ON ENRICHMENT OF SELENIUM IN TOBACCO FROM SOME AREA OF CHINA

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Introduction

As we know, smoking is one of hobbies for people, but it is harmful to human health. Many epidemiological studies have demonstrated that smoking is one of main causes leading to lung cancer, and tbe incidence of lung cancer varies with country and area (Bogder *et al*, 1981). Chortyk *et al* (1984) found that Se could restain the toxicity of cigarette tar. But, to date, some problems remain unclear. The primary objective of this study is to explore Se status of natural tobacco and various kinds of cigarette, factors affecting Se content of tobacco, and effect of the natural organic Se content of cigarette on tar, free radical content and Se level and activity of glutathion peroxidase of human and animals blood.

Materials and Methods

139 samples of tobacco and 86 samples of different kinds of cigarette, taken from different areas of China, are selected for the study. Cigarette samples are simulately smoked using SM-302 smoking machine, and Se in main airflow of tobacco fume are collected with Cambridge glass filter. Total Se content in tobacco, cigarette, tobacco fume and serum are determined by fluorescence spectrophometry. Cigarette tar content is determined using SM-302 smoking machine. Free raclical is determined by JES- FELXG paramagnetic resonance.

Results and Conclusion

Results show that there is Se enrichment in some natural tobacco and cigarette samples, but most samples have low Se content. The statistical result has showed that Se content varies with tobacco samples from 0.0043 to 0.690 Se mg/kg with a geometric mean of 0.172 Se mg/kg, 62.5% of samples are low Se content (less than 0.143 Se mg/kg), and 10.07% of samples are high Se content (more than 2.40 Se mg/kg). For cigarette samples, Se content varies from 0.0086 to 0.302 Se mg/kg, their geometric mean is 0.160 Se mg/kg, 50 % of those samples have Se content less than 0.160 mg/kg, only 4.65% of samples have more than 0.420 Se mg/kg. Se content in tobacco is greatly affected by soils of tobacco garden. Positive correlation is found between Se in tobacco and that in soil, their correlation equation is: Y (Se in tobacco) 0.230 + 0.244 x (Se in soil) ($r = 0.9219^{**}$). High Se tobacco originated mainly from soils which developed on high Se rock, such as siliceous shale of Permian period, and low Se tobacco is produced on soils derived from calcareous slate, limestone, argillaceous shale and loess.

Simulate test indicates that Se content of main cigarette fume is 8 to 11% of total Se contention in cigarette, and Se content of cigarette has great effect on cigarette tar and free radical content. Significant negative correlation are found between Se content of cigarette and tar, free radical content, their correlation equations are:

Y (tar content) = -3.35X(Se in cigarette) + 24.20 ($r = -0.9966^{**}$)

Y (free radical content) = -3.019×10^{13} X(Se in cigarette) + 10.10×10^{13} ($r = -0.9408^{**}$)

Animal test show that Se content of cigarette has significant affection Se level of serum and glutathion peroxidase activity of rabbit. For smoking treatment of high Se cigarette (1.356 Se mg/kg), blood Se increase rate reached 12.0% (significant level), and increase rate of glutathion peroxidase activity is 8.6% (significant level). But for another two treatments (low Se cigarette, 0.220 Se mg/kg, and control, on smoking), both increase rate of both blood Se content and glutathion peroxidase activity are insignificant.

Se content of cigarette also has slightly effect on Se level of human blood. After end of test, Se content of blood of smoker who smoke high Se cigarette (1.336 Se mg/kg) increase about 5.55%, but increase rate of blood Se content varies with age, maximum (6.5%) for 31 to 40 year old group and minimum (1.1%) for 61 to 70 year old group. It is suggested that high Se cigarette is helpful to replenish Se to smokes and restain the toxicity of cigarette tar.

CRITERIA OF ENVIRONMENTAL-GEOCHEMICAL ASSESSMENT OF METALLURGICAL CENTRES IN RUSSIA AS RELATED TO THE RISK OF MICROELEMENTOSES AND CANCER

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The incidence of microelementoses diseases and of respiration organs cancer by population is an important criterion for assessing the status of the environment polluted by metallurgical enterprises. These diseases and their reasons are the following: haemochromatosis and hepatosis - Fe, non-specific hypercupremias - Cu, manganoses - Mn, chromic hepatosis - Cr, heart aluminosis - Al, etc. The spatial pattern of diseases incidence may indicate zones (individual loci) of environmental constraint and even disaster.

Carcinogenic-landscape-geochemical investigation in the Southern Urals (Cheliabinsk and Orenburg region) - old industrial region with ferrous and non-ferrous metallurgy, machine building and metal processing industries, revealed the highest risk for population in large industrial centres (Magnitogorsk, Orsk, Novotroitsk). Lung cancer is predominant because of considerable atmospheric input of predominant carcinogenic pollutants (heavy metals, polycyclic aromatic hydrocarbons) into environment (including human organism). The standardized indices of lung cancer incidence in Magnitogorsk were as follows: 96.5 for men and 20.6 for women, i.e. they are by 1.5 and 2.5 times higher than mean parameters for CIS. Intensive indices for the same disease surpass the mean ones by 2.3 and 3.3 times, respectively. The risk for men is by 4.7 times higher than for women due to their jobs: 44.4 of men are engaged in metallurgical enterprises (there are only 16.9 of women there); of minor importance seems to be the habit of smoking proper to men, more than women. The problem is especially urgent, because almost half of the sick persons did not reach their pension age.

In order to reveal the ratio between the disease incidence caused by environmental and occupational reasons, only the population, that was not working on metallurgical plants was the object of study. The lung cancer incidence equals 72.6 for men and 39.8 for women living in the ecologically dangerous part of the town, whereas in the rather "clean" one these parameters were 31.3 and 8.9. The dynamics for 25 years proves the increase of risk both for men, and for women. The frequency of children incidence in the town is the highest for respiratory organs: 54 to 65% (influenza excluded) of the total. The incidence is evidently higher in the regions with unfavourable environmental conditions.

A reliable positive correlation has been found for: a) the lung cancer incidence among grown-up persons and technogenic loading on the environment (P_c = 0.97. $0.5 < P > 0.1$); b) children incidence (acute infections of the upper respiratory tract, permanent bronchite) and rate of technogenic Ioading ($P_c = 0.95$, $0.5 < P > 0.1$).

Mean standard parameters for Cheliabinsk region of the lung cancer incidence are the following: 63.6 for men and 14.1 for women. The carcinogenous structure is the same as the average for the country and may be presented as the following decreasing sequences. For men: lung cancer (28%) > gastric cancer (23%) > skin cancer (11%); the lung cancer incidence is on the fifth place among women after those of gastric - (18.1%), skin - (16.5%), cervix of the uterus (12.4%), breast - (11.7%) cancer.

Areas with very high cancer incidence and its quick growth are specified within the Magnitogorsk region. Such is the town of Plast with adjacent area and Chebarcul region. Towns of Zlatoust, Karabash with their vicinties, Kislinsk region have extremely high cancer incidence indices: 333 to 444 (men) and 259-370 (women) practically for all cancer sites. Thus, the intensive index of

lung incidence is 99-121 for men and 24-32 for women. This demonstrates the possibility to reveal the hazard zone by epidemiologic investigations data.

It may be assumed now that the increase of disease incidence during the last 15 years originates from the radioactive "Ural trace" exerting either a direct or an direct effect on the human organism.

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